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TUBERCULOSIS CONTROL ISSUE NO. 24

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Complement Fixation for Histoplasmosis I, II

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# Public Health Reports

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## EDITORIAL

### DECLINE OF TUBERCULOSIS MORTALITY

It is a provocative fact that since Koch's discovery of the causative organism, the mortality of tuberculosis has consistently diminished. It is, indeed, almost as if the mere exposure of the *mycobacterium tuberculosis* in its insidious role had robbed it of some of its sting. As an explanation of the phenomenon of declining mortality, however, this is of course idle sophistry. We must therefore look elsewhere for feasible explanation.

The possibility that the human strain of the tubercle bacillus has suffered a decline in virulence is open to serious question as well; indeed, there is little evidence to admit of such a conjecture. It appears more likely that this decreasing mortality rate arises rather from some important change in the parasite's human host—or, to be more exact, some complex of change, of which the host's environment probably plays a part.

It is conceivable, for example, that the forces of natural selection have, over the course of centuries, operated in such a fashion as now to confront the tubercle bacillus with a more resistant host. Furthermore, improved socio-economic conditions, in this country at least, are believed by many students of the problem to have played a significant role, although the evidence here is largely presumptive.

Whether these factors are entirely responsible for the phenomenon of declining mortality, or whether other causative influences can be held more cogently related, is, of course, not precisely determinable at the present time. Nor is it easy to determine the exact influence exerted by the deliberate efforts which have been undertaken to control the disease since Koch made his historic discovery. Certainly, the declining death rate has accompanied the development and applica-

\*This is the twenty-fourth of a series of special issues of PUBLIC HEALTH REPORTS devoted exclusively to tuberculosis control, which will appear the first week of each month. The series began with the March 1, 1946, issue. The articles in these special issues are reprinted as extracts from the PUBLIC HEALTH REPORTS. Effective with the July 5, 1946, issue, these extracts may be purchased from the Superintendent of Documents, Government Printing Office, Washington 25, D. C., for 10 cents a single copy. Subscriptions are obtainable at \$1.00 per year; \$1.25 foreign.

tion of control measures; and it is noteworthy that even those areas deficient in control facilities have shared in the general decline, a fact for which several valid explanations have been advanced.

Whatever the specific causes of the current, salutary trend, however, and whatever their relative individual weight in the total process, it is a preeminent fact that current statistical data on tuberculosis mortality rates are not an index of current infection and morbidity. Nor, in fact, are they a measure of the effectiveness of current control efforts. They are merely the expression of the relative condition or state of these factors at some time in the recent past. In similar fashion, we may assume that the tuberculosis death rate of the future will reflect the present universe of environmental control and human resistance and susceptibility.

It becomes strikingly plain, then, that we today, to the extent that our epidemiological principles of control are sound, hold the key to future mortality in this country. We possess a priceless advantage, in that certain forces have converged to render tuberculosis vulnerable at this time. If we are to confirm and assure for the future the present encouraging trend of tuberculosis mortality—if, indeed, we are to hope to eliminate tuberculosis as a public health problem—we are duty-bound to effect the widest possible extension of controls now, when conditions appear to favor the success of our efforts. For if it is true that tuberculosis began its retreat during the infancy of a control movement in this country, may we not expect it to suffer ultimate defeat under the onslaught of a mature, well-integrated, and progressive control program?

One further factor renders the immediate prosecution of control measures urgent. Since we do not with certainty know the precise influence of the elements which have in the past affected tuberculosis mortality, we have no assurance that these elements will in the future conspire in the same way to contribute positively to its decline. There is no assurance, in short, that our present advantage is not temporary, for it is entirely within the realm of possibility that those factors over which we have no direct control may themselves undergo natural or artificial change. Lest such changes take the direction of the current epidemiological trend from our hands irrevocably, then, affirmative action must be taken promptly to consolidate and extend our tuberculosis control program in all areas of activity.

FRANCIS J. WEBER, *Medical Director,*  
*Chief, Tuberculosis Control Division*



# A COMPLEMENT FIXATION TEST FOR HISTOPLASMOSIS

## I. Technic and Preliminary Results on Animal Sera<sup>1</sup>

By: DANIEL J. TENENBERG, *Bacteriologist and ARDEN HOWELL, JR., Senior Mycologist, United States Public Health Service*

A serological test for human histoplasmosis would be a valuable adjunct in the diagnosis of the disease and in the interpretation of the histoplasmin skin reaction. No references to such a test have been noted in the literature. The complement fixation test described in the present paper was devised to fit this need.

The first problem in the development of a complement fixation test is the selection of a proper antigen. Such an antigen should detect antibodies in infected animals, and antibodies reacting with this antigen should not be present in the serum of normal animals.

**Materials and Methods.**—Preliminary complement fixation studies were carried out using histoplasmin (lot H-15—1:100) as the antigen and the sera of experimentally inoculated guinea pigs as the source of antibodies.

The test conforms essentially with the Kolmer complement fixation test for syphilis (1) with the exception of the nature and volume of antigen and the volume of serum employed.

The materials and methods employed are as follows:

1. Histoplasmin antigen (2): 0.2 cc. of 1:100 dilution of lot H-15.
2. Serum: 0.2 cc. of undiluted and serial two-fold dilutions.
3. Guinea pig complement: 1.0 cc. containing 2 full units. The complement titration is performed in the presence of a test dose of the antigen.
4. Washed, fresh sheep red blood cells: 0.5 cc. of a 2 percent saline suspension.
5. Anti-sheep rabbit hemolysin: 0.5 cc. containing 2 units.
6. Saline: 0.85 percent C. P. NaCl in distilled water.
7. Kolmer test tubes, chemically clean.

A typical protocol is shown in table 1. It will be noted that the first four control tubes do not contain serum, and that three tubes are employed for a given dilution of each serum to be tested.

TABLE 1.—Sample complement fixation protocol

Tube	Volume in cc.						Cells	Purpose
	Serum	Antigen (histoplasmin 1:100)	Complement (2 full units)	Hemolysin (2 units)	Saline			
1	-----	-----	-----	-----	2.5	Mix. Incu-	0.5	Cell suspension control.
2	-----	-----	1.0	0.5	1.0	bate 6-8°	0.5	Hemolytic system control.
3	-----	0.2	1.0	0.5	0.8	C. for 15-18 hours	0.5	Antigen anti-complementary control.
4	-----	0.2	-----	-----	2.3	followed by 15' in water bath at 37° C.	0.5	Hemolytic control of antigen.
5	0.2	-----	-----	-----	2.3		0.5	Hemolytic control of serum.
6	0.2	-----	1.0	0.5	0.8		0.5	Anti-complementary control of serum.
7	0.2	0.2	1.0	0.5	0.6		0.5	Test.

<sup>1</sup> From the Office of Field Studies, Tuberculosis Control Division.

tion of control measures; and it is noteworthy that even those areas deficient in control facilities have shared in the general decline, a fact for which several valid explanations have been advanced.

Whatever the specific causes of the current, salutary trend, however, and whatever their relative individual weight in the total process, it is a preeminent fact that current statistical data on tuberculosis mortality rates are not an index of current infection and morbidity. Nor, in fact, are they a measure of the effectiveness of current control efforts. They are merely the expression of the relative condition or state of these factors at some time in the recent past. In similar fashion, we may assume that the tuberculosis death rate of the future will reflect the present universe of environmental control and human resistance and susceptibility.

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# A COMPLEMENT FIXATION TEST FOR HISTOPLASMOSIS

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3. Guinea pig complement: 1.0 cc. containing 2 full units. The complement titration is performed in the presence of a test dose of the antigen.
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	Serum	Antigen (histoplasmin 1:100)	Complement (2 full units)	Hemolysin (2 units)	Saline			
1	—	—	—	—	2.5	Mix. Incubate 6-8° C. for 15-18 hours followed by 15' in water bath at 37° C.	0.5	Cell suspension control.
2	—	—	1.0	0.5	1.0		0.5	Hemolytic system control.
3	—	0.2	1.0	0.5	0.8		0.5	Antigen anti-complementary control.
4	—	0.2	—	—	2.3		0.5	Hemolytic control of antigen.
5	0.2	—	—	—	2.3		0.5	Hemolytic control of serum.
6	0.2	—	1.0	0.5	0.8		0.5	Anti-complementary control of serum.
7	0.2	0.2	1.0	0.5	0.6		0.5	Test.

<sup>1</sup> From the Office of Field Studies, Tuberculosis Control Division.

Four-plus complement fixation was considered positive; three- or two-plus, doubtful; and one-plus or no fixation, negative.

In the anticomplementary titration, undiluted histoplasmin antigen (lot H-15) was not anticomplementary. In this titration the complement employed had previously been titrated in the absence of the antigen.

*Complement fixation tests with the blood of experimentally inoculated guinea pigs.*—In order to determine if histoplasmin (lot H-15) is a satisfactory antigen in the complement fixation test, a number of sera from twelve guinea pigs inoculated with viable *Histoplasma capsulatum* (1:100 saline suspension of the yeast phase injected intraperitoneally) were collected. These sera were not obtained earlier than 7 months after the inoculation of the animals. The sera of 9 of 12 experimentally inoculated guinea pigs showed some degree of complement fixation. Five of these sera gave complete fixation, four gave fixation complicated by some anticomplementary effect of the sera and three gave no fixation. These data are sufficient to show that complement-fixing antibodies to histoplasmin (1:100 lot H-15) are produced in guinea pigs inoculated with *Histoplasma capsulatum*.

*Complement fixation tests with the blood of non-infected guinea pigs.*—In order to rule out the presence of complement-fixing substances in normal sera, "normal" guinea pigs were bled. Sera obtained from 33 of these "normal" guinea pigs were all negative. Eight of these "normal" guinea pigs had been skin-tested repeatedly with histoplasmin and blastomycin with consistently negative skin-test reactions. The implication is that repeated skin tests with histoplasmin, in the dilutions employed, do not produce complement fixing antibodies to histoplasmin in uninoculated or uninfected animals.

*Cross-reactions in the complement fixation test when histoplasmin and blastomycin are used as antigens.*—Since positive blastomycin skin reactions have been described in *Histoplasma*-inoculated guinea pigs whose histoplasmin skin reactions were positive (2, 3) it seemed desirable to investigate possible cross-reactions between these two antigens by the serological method.

A complement fixation test, employing blastomycin as the antigen, had to be devised. Merely changing the antigen in the above-described complement fixation test, substituting a 1:100 dilution of blastomycin (lot B-7) for histoplasmin, was satisfactory. Undiluted blastomycin was not anticomplementary. With this test, employing sera from thirteen guinea pigs which had been inoculated with viable *Blastomyces dermatitidis*, six sera gave some degree of complement fixation. Of these six sera which fixed complement, three gave four-plus fixation and three gave two-plus fixation. Control sera of 19 "normal" guinea pigs gave no complement fixation with blastomycin.

First, to investigate cross-reactions, complement fixation tests were performed in which were employed a 1:100 dilution of histoplasmin (lot H-15) and blastomycin (lot B-7) as antigens. The sera of three guinea pigs which had been inoculated with *Histoplasma*, and which had given four-plus fixation with histoplasmin, were chosen as the source of antibodies. The titers of these sera, with the two above antigens, are shown in table 2. As in the skin tests, the complement fixation test revealed cross-reactions. However, as in the skin tests (2), the complement fixation test with the homologous antigen (histoplasmin 1:100, lot H-15) was positive in higher serum titer than with the heterologous antigen (blastomycin 1:100, lot B-7), and the cross-reactions were eliminated by diluting the serum (table 2).

TABLE 2.—Cross-reactions in the complement fixation test employing a 1:100 dilution of histoplasmin (lot H-15) and blastomycin (lot B-7) as antigens and the sera of *Histoplasma*-inoculated guinea pigs

Serum guinea pig No.	Undiluted		Serum dilution							
			1:2		1:4		1:8		1:16	
	H-15	B-7	H-15	B-7	H-15	B-7	H-15	B-7	H-15	B-7
177	++++	++	++++	0						
168	++++	++++	++++	++++	++++	+				
191	++++	++++	++++	++++	++++	+++	++++	+	++++	0

H-15—Histoplasmin.

B-7—Blastomycin.

++++—Complete complement fixation.

0—No complement fixation.

Next, the histoplasmin (1:100 lot H-15) was tested with the six *Blastomyces* animals' sera which had shown complement-fixing antibodies for blastomycin. Of the three sera which produced four-plus fixation with blastomycin, one gave a two-plus fixation, and two gave no fixation with histoplasmin. None of the three sera which gave two-plus fixation with blastomycin gave any fixation with histoplasmin.

In order further to investigate the cross-reactions, various dilutions of the two antigens were tested against the pooled sera of guinea pigs which had been inoculated with *Histoplasma* and the pooled sera of guinea pigs which had been inoculated with *Blastomyces* (table 3).

Table 3 shows that the homologous antigen gives fixation in higher dilutions of serum than does the heterologous antigen. It also shows that a 1:100 dilution of either antigen is close to the minimal concentration of antigen necessary for fixation. Serum dilutions apparently are more effective than antigen dilutions in the elimination of cross-reactions.

The results of these experiments on cross-reactions indicate that with the two antigens studied the homologous reactions are stronger than the heterologous reactions. This is similar to the results of skin test cross-reactions (2). Further, they indicate that these two antigens, in a 1:100 dilution, are comparable in combining power in their respective homologous reactions.

TABLE 3.—Results of complement fixation tests with various dilutions of pooled sera of *Histoplasma*-inoculated guinea pigs and pooled sera of *Blastomyces*-inoculated guinea pigs employing histoplasmin (H-15) and blastomycin (B-7) in various dilutions as antigens

HISTOPLASMIN DILUTIONS					
H-serum <sup>1</sup>	1:100	1:200	1:400	1:800	1:1600
1:2	++++	++	+	0	0
1:4	++++	0	0	0	0
1:8	++++	0	0	0	0
1:16	++	0	0	0	0
BLASTOMYCIN DILUTIONS					
H-serum <sup>1</sup>	1:100	1:200	1:400	1:800	1:1600
1:2	++++	++	0	0	0
1:4	+	0	0	0	0
1:8	0	0	0	0	0
1:16	0	0	0	0	0
HISTOPLASMIN DILUTIONS					
B-serum <sup>2</sup>	1:100	1:200	1:400	1:800	1:1600
1:2	AC	AC	AC	AC	AC
1:4	+	+	+	0	0
1:8	0	0	0	0	0
BLASTOMYCIN DILUTIONS					
B-serum <sup>2</sup>	1:100	1:200	1:400	1:800	1:1600
1:2	AC	AC	AC	AC	AC
1:4	++++	++	0	0	0
1:8	++++	+	0	0	0

<sup>1</sup> H-serum indicates pooled sera from *Histoplasma*-inoculated guinea pigs.

<sup>2</sup> B-serum indicates pooled sera from *Blastomyces*-inoculated guinea pigs.

++++ Complete complement fixation.

0 No complement fixation.

AC Anti-complementary.

#### DISCUSSION

The purpose of these investigations was to develop a complement fixation test for human histoplasmosis. In such a technic, the selection of a satisfactory antigen is the primary problem. It has been shown that a 1:100 dilution of lot H-15 histoplasmin, a culture filtrate of *H. capsulatum*, appears to be such an antigen, and that complement-fixing antibodies which will combine with this antigen are present in the sera of guinea pigs experimentally inoculated with *Histoplasma capsulatum* 7 months before the sera were obtained. No such complement-fixing antibodies are found in normal guinea pig sera.

Similarly, complement-fixing antibodies against lot B-7 blastomycin, a culture filtrate of *B. dermatitidis*, were found in the sera of guinea pigs which had been experimentally inoculated with *Blastomyces dermatitidis* several months previous to the time serum was obtained. Martin (4) has described a complement fixation test in blastomycosis, in which a saline suspension of *Blastomyces* is used as the antigen.



In both tests, cross-reactions between blastomycin and histoplasmin have been noted, as well as in the skin tests (2,3) in which these same antigens were employed. While it is obvious that these two antigens are closely related antigenically, in both skin and serological tests the homologous reactions were found to be stronger than the heterologous reactions. In the skin test it has been demonstrated (2) that if the critical titer of either antigen is employed, the percentage of cross-reaction is considerably reduced, and the antigens are relatively specific. In the complement fixation tests, it has been shown that the homologous reaction can be differentiated from the heterologous by the method of serial serum dilutions, as is shown in tables 2 and 3.

The results of the experiments reported above, which show some degree of cross-reaction between histoplasmin and blastomycin with sera of guinea pigs experimentally inoculated with either *Histoplasma capsulatum* or *Blastomyces dermatitidis* are not in agreement with the studies of Martin (4) who, in describing a complement fixation test for human blastomycosis, reported no cross-reaction with a crude suspension of *Histoplasma* tested with sera from three patients with blastomycosis. This may have been due to the fact that the antigen used by Martin was weaker in combining power than the histoplasmin (1:100 dilution of lot H-15) used in the experiments reported above. It has been shown, for example, (2) that various lots of histoplasmin differ considerably in their potency as skin-test antigens.

As previously noted, only the sera of 9 of 12 guinea pigs inoculated with *Histoplasma* showed complement-fixing antibodies for a 1:100 dilution of histoplasmin (lot H-15). However, the first sera from these experimental animals were not obtained until approximately 7 months after inoculation. Similarly, only the sera of 6 of 13 guinea pigs inoculated with *Blastomyces* showed complement-fixing antibodies for a 1:100 dilution of blastomycin (lot B-7). Perhaps a greater percentage of positive serological reactions would have been obtained had the animals been bled earlier. Experiments should be performed to elucidate this relationship of stages of infection, skin reactions, and serology.

It is possible that the complement-fixing antibodies differ from the substances which confer sensitivity to the skin. Also, positive serology may be subject to an interpretation different from that attached to skin sensitivity. It may be, for example, that serology in contrast to skin tests will be a means of differentiating between active, or recent, extensive infection on the one hand and inactive, or long-past infection on the other. There is some evidence to support this, in the tests performed on human sera (5).

A more concentrated antigen preparation might prove to be more satisfactory than the 1:100 dilution of antigen employed in the



present paper. A 1:100 dilution of histoplasmin (H-15) and of blastomycin (B-7) represents approximately one antigenic unit. Since undiluted preparations of these antigens are not anticomplementary, more concentrated preparations than a 1:100 dilution can be employed. Also, purification and/or concentration of the active antigenic material in histoplasmin might result in stronger reactions with fewer cross-reactions.

#### SUMMARY

1. A complement fixation test for histoplasmosis is described. Preliminary results indicate that lot H-15 histoplasmin is a satisfactory antigen for detecting complement-fixing antibodies in guinea pigs experimentally inoculated with *Histoplasma capsulatum*.

2. Similarly, a complement fixation test for blastomycosis is described. Lot B-7 blastomycin is a suitable antigen to demonstrate complement-fixing antibodies in guinea pigs experimentally inoculated with *Blastomyces dermatitidis*.

3. Complement-fixing antibodies against both antigens may be present in the serum of *Histoplasma*- or *Blastomyces*-inoculated guinea pigs. However, fixation is stronger with the homologous than the heterologous antigen, and cross-reactions can be eliminated by serial serum dilutions.

4. Normal guinea pig sera do not give positive complement fixation with histoplasmin or blastomycin.

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- (3) Olson, B. J., Bell, J. A., and Emmons, C. W.: Studies of the role of fungi in pulmonary disease. I. Cross-reactions of histoplasmin. Pub. Health Rep., **60**: 1383-1394 (1945).
- (4) Martin, D. S.: Complement fixation in blastomycosis. J. Infect. Dis. **57**: 291-295 (1935).
- (5) Furcolow, M. L., Bunnell, I. L. and Tenenberg, D. J.: A complement fixation test for histoplasmosis. II. Preliminary results with human sera. This issue, p. 169.

#### NOTE

After manuscript for this article had gone to press, the authors noted the two references to complement fixation tests for histoplasmosis listed below:

- (1) Salvin, S. B.: Complement fixation studies in experimental histoplasmosis. Proc. Soc. Exp. Biol. and Med. **66**: 342 (Nov. 1947).  
The paper reports details indicating that highly specific results are obtained in rabbits and humans using antigen from the yeastlike phase of *H. Capsulatum*.
- (2) Miller, H. E., Keddie, S. M., Johnstone, H. G., and Bostick, W. L.: Histoplasmosis. Cutaneous and mucomembranous lesions, mycologic and pathologic observations. Arch. Derm. and Syph. **56**: 715-739 (Dec. 1947).  
This paper reports a positive complement fixation test on the serum of one proved case. No data on controls are included.

## A COMPLEMENT FIXATION TEST FOR HISTOPLASMOSIS

### II. Preliminary Results with Human Sera<sup>1</sup>

By: MICHAEL L. FURCOLOW, *Surgeon*, IVAN L. BUNNELL, *S. A. Surgeon* and DANIEL J. TENENBERG, *Bacteriologist*, *United States Public Health Service*

The preceding article in this issue (1) describes a complement fixation test by means of which it was possible to demonstrate that antibodies which combine with histoplasmin occur in the sera of *Histoplasma*-inoculated guinea pigs and are absent from the sera of guinea pigs which had not been inoculated. The present paper reports the use of this test with human sera.

#### MATERIAL AND METHODS

The technic of the complement fixation test is similar to the Kolmer test for syphilis (2). Observed results are expressed in the customary notation of 4-plus, 3-plus, 2-plus, 1-plus and 0 (negative) fixation but for the purposes of the present paper, the results are summarized in 3 groups: *Positive*, complete, 4-plus, fixation; *Doubtful*, 3- and 2-plus fixation; *Negative*, 1-plus and 0 fixation. For a few persons tested, more than one serum was available and it is evident that some variation exists in the results obtained from repeated tests on sera drawn at different times from the same individual. Sufficient data for a study of variation in the degree of fixation, or changes in fixation with time, are not as yet available. Except where noted, the results reported in this paper are the highest degree of fixation observed.

The histoplasmin used as the antigen for the complement fixation test is the same, lot H-15, as has been used extensively for intradermal testing of human beings (3) (4). It has been titered for specificity and potency in experimentally inoculated guinea pigs (5).

Intradermal tuberculin and histoplasmin tests were done on a number of the persons whose complement fixation tests are reported here. The tuberculin (PPD-S) was furnished by Dr. Florence Seibert of the Henry Phipps Institute of Philadelphia, Pennsylvania. A dose of 0.0001 mg., in 0.1 cc. of diluent, was used. Histoplasmin skin tests were made with 0.1 cc. of a 1:1000 dilution of lot H-15. Reactions to both tuberculin and histoplasmin were considered positive if the induration measured 5 or more mm. in diameter at the 48-hour reading.

The 300 persons whose sera were tested were all residents of Missouri and Kansas, the majority of the Kansas City area. They were chosen to furnish an estimate of the frequency of the occurrence of complement-fixing antibodies in the sera of selected groups. Insofar as material was available for study, an attempt was made to test proved

<sup>1</sup> From the Office of Field Studies, Tuberculosis Control Division.

cases of histoplasmosis, various groups in which infection might be suspected, and groups in which, a priori, infection would not be expected.

Table 1 shows the frequency of positive, doubtful, and negative complement fixation tests in four main groups of cases. Altogether, 34 or 11.4 percent of the sera of the total group of 300 persons showed the presence of complement-fixing antibodies. Fourteen, or 4.7 percent of the tests were classified as being positive, while 20 or 6.7 percent were designated as doubtful.

TABLE 1.—Frequency of positive, doubtful and negative complement fixation tests in 4 groups of cases

Group (see text for description)	Number in group	Positive (4-plus tests)		Doubtful (3- and 2-plus tests)		Negative (1-plus and 0 tests)	
		Number	Percent	Number	Percent	Number	Percent
Proved cases of histoplasmosis.....	9	6	66.7	2	22.2	1	11.1
Histoplasmin "converters".....	13	2	15.4	1	7.7	10	76.9
Histoplasmin-positive, tuberculin-negative persons with unhealed pulmonary lesions (4).....	36	6	16.7	4	11.1	26	72.2
Miscellaneous "control" cases.....	242	0	0	13	5.4	229	94.6
Total.....	300	14	4.7	20	6.7	266	88.6

*Proved cases of histoplasmosis.*—In the course of our work in Kansas City, it has been possible to obtain sera from 9 proved cases of histoplasmosis. *Histoplasma capsulatum* was isolated by cultures from 8 patients; in the 9th, (J. W.) cultures were not taken but it was possible to confirm the diagnosis by microscopic study of the parasitized liver, spleen and adrenal gland. Further details of these cases are given in table 2 which gives results of complement fixation tests, histoplasmin tests, and a notation as to whether the case was living or dead at the time of this report.

TABLE 2.—Results of intradermal histoplasmin tests, complement fixation tests and related data on 9 proved cases of histoplasmosis<sup>1</sup>

Case	Status	Histoplasmin skin test	Tested	Number of sera—Degree of complement fixation				
				4+	3+	2+	1+	0
CD.....	Living.....	Positive.....	3	3				
WB.....	do.....	do.....	6	4		1	1	
SP.....	do.....	do.....	3	2	1			
GM.....	do.....	do.....	3	3				
JG.....	Dead.....	do.....	4	3		1	1	
FT.....	do.....	do.....	4	2	1		1	
EW.....	do.....	Negative.....	1					1
PP.....	do.....	do.....	2		1		1	
JW.....	do.....	do.....	1	1				

<sup>1</sup> For further description of these cases see (6).

<sup>2</sup> This serum showed some anticomplementary effect.

<sup>3</sup> One serum showed some anticomplementary effect.

According to the results shown, complement fixation, either 4-plus or 3-plus, was obtained on at least one serum from 8 of the 9 cases. There is some indication of a correlation between results of the skin and the complement fixation tests—6 of the 9 had positive reactions to both histoplasmin and complement fixation tests and the one person negative to the complement fixation test was also one of the 3 non-reactors to histoplasmin. In the complement fixation test, the results with sera from human cases closely parallel those obtained with guinea-pigs inoculated with *Histoplasma* (1).

*Histoplasmin "converters".*—Periodic skin testing with histoplasmin has been carried out during the past year on a large group of Kansas City school children. In the course of this work definite changes from negative to positive histoplasmin skin reactions, similar to tuberculin conversions, have been observed. Since changes in the tuberculin reaction from negative to positive are generally agreed to be a consequence of tuberculous infection, it may be postulated that histoplasmin conversion is a result of recent infection with *Histoplasma*.

At the present time a number of children—histoplasmin converters—are being observed. Sera from 13 such cases, whose conversion occurred 7 to 9 months before drawing the sera, were tested for the presence of complement-fixing antibodies. Two of the 13 showed definite and one doubtful complement fixation. These results, though observed at a time after conversion which may not be optimal for the demonstration of antibodies, show that the recent acquisition of skin sensitivity to histoplasmin may be associated with the same type of serological response as is observed in proved *Histoplasma* infections. Further intensive studies of these cases are in progress. It is significant that none of the 13 show clinical evidence of disease.

*Histoplasmin-positive, tuberculin-negative persons with unhealed pulmonary lesions.*—Sera were obtained from a special group of 36 children and adults who form part of a larger group which has been under intensive observation in connection with our studies in Kansas City. These 36 individuals are characterized by the fact that when first discovered, they were sensitive to histoplasmin but not to tuberculin and that their chest roentgenograms showed the presence of otherwise undiagnosed, persistent, unhealed pulmonary lesions. Careful follow-up studies of persons showing these characteristic lesions indicate slow healing by calcification. Descriptions and illustrations of these lesions are given in an earlier publication (4). Although the evidence is not yet conclusive, it is believed that these cases represent mild, subclinical infections with *Histoplasma capsulatum*. As shown in table 1, 6 of the 36 individuals had positive complement fixation tests and 4 had doubtful tests. Thus the same antibodies found in 8 of the 9 proved cases have been demonstrated in 10 of these 36 suspected cases.

*Miscellaneous, "control," cases.*—In order to obtain information about the specificity of the complement fixation reaction and to determine the presence of complement-fixing antibodies in "control" individuals, including persons with various diseases, 242 sera were tested. Seventy of these sera were from consecutive general hospital admissions, drawn for routine Kolmer and Kahn tests. Ninety-one sera were from patients in a tuberculosis sanatorium for whom the diagnosis was proved by isolation of tubercle bacilli. An additional 81 sera were from persons referred to us for a variety of reasons. Fifty-eight individuals in this last subgroup were histoplasmin-positive, tuberculin-negative; 23 were negative to both histoplasmin and tuberculin. No positive (4-plus) complement fixation tests were found in the total group of 242 individuals. Altogether, 13 doubtful tests were observed: 7 among the 91 tuberculous patients, 6 among the 58 histoplasmin reactors, none among the 70 general hospital admissions, or the 23 histoplasmin nonreactors.

*Cross-reactions with blastomycin.*—Complement fixation tests were also performed on all sera using blastomycin as the antigen. As reported in the tests with guinea-pig sera (1), cross-reactions may also occur with human sera. However, in most cases there was less complement fixation with blastomycin than with histoplasmin. These findings will be reported later.

#### DISCUSSION AND SUMMARY

The present paper reports the results of complement fixation tests for histoplasmosis on 300 human sera. The specificity of the test, using histoplasmin, lot H-15, as the antigen was previously shown with guinea-pig sera: *Histoplasma*-inoculated animals showed the presence, and uninoculated animals the absence, of complement-fixing antibodies (1).

Fourteen positive complement fixation tests were found among the 300 human sera tested. All 14 were found in a relatively small group of 58 persons: 9 definitely proved cases of histoplasmosis, 13 persons with recently acquired skin sensitivity to histoplasmin and 36 persons with unhealed lung lesions characteristic of those associated with histoplasmin sensitivity. Seven, or 12 percent, of these 58 sera showed doubtful complement fixation tests. No positive tests were found among the remaining 242 "control" human sera; 13, or 5 percent, showed doubtful tests.

It is too early to draw definite conclusions on the interpretation of the complement fixation test and its ultimate usefulness. However, it may well be that the test will serve as an extremely important link in the chain of evidence connecting *Histoplasma* infection, sensitivity to histoplasmin, and chronic lung lesions which heal by calcification.



## ACKNOWLEDGMENT

The authors desire to express their appreciation to Carroll E. Palmer, Senior Surgeon, for continued advice and encouragement.

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## THE EFFICIENCY OF METHODS FOR THE ISOLATION OF *HISTOPLASMA CAPSULATUM*<sup>1</sup>

By: ARDEN HOWELL, JR., PH. D., *Senior Mycologist United States Public Health Service*

## INTRODUCTION

Numerous methods have been employed in attempts to isolate *Histoplasma capsulatum* from tissues (1-7). In order to determine a satisfactory procedure to be used, especially for materials in which the organisms are rare, the following study was undertaken.

## MATERIALS AND METHODS

The strain of *Histoplasma capsulatum* used in the study was obtained from Dr. Norman F. Conant, Duke University Medical School, who reported that it was isolated in 1944 from a case of histoplasmosis in South Africa.

Seventy-nine guinea pigs were employed. Each of these was experimentally infected by the intraperitoneal injection of graded doses of a saline suspension of the yeast phase of *Histoplasma capsulatum*. The animals were then sacrificed at intervals. At autopsy, the spleen of each was removed and a portion of each cultured on each of two plates of blood agar and two of potato dextrose agar.

<sup>1</sup> From the Office of Field Studies, Tuberculosis Control Division.

The blood agar was prepared from Difco brain-heart infusion to which was added 2 percent Bacto agar and 10 percent sterile defibrinated horse blood, and will be termed B. H. I. Blood Agar or blood agar plates in this paper. The potato dextrose agar was prepared according to the method previously described (8). Streptomycin and penicillin, in a concentration of 40 and 20 units, respectively per ml. of medium, were added, as recommended by Thompson (9). After inoculation, all plates were sealed with sterile vaseline. One plate of each medium was then incubated at room temperature and the other at 37° C.

All colonies, suspected of being *Histoplasma*, were subcultured on potato dextrose agar slants which were incubated at room temperature.

## RESULTS

Of the 79 guinea pigs used in this study, positive cultures were obtained from the spleens of 46. The media on which these cultures were obtained, and the temperatures at which the cultures had been maintained, are shown in table 1.

TABLE 1.—Comparison of results of cultures on potato dextrose agar<sup>1</sup> at room temperature and on B. H. I. blood agar at room temperature and at 37° C.

Potato dextrose agar plates at room temperature	B. H. I.—Blood Agar Plates								
	Positive at room temperature			Negative at room temperature			Total		
	Posi- tive 37° C.	Nega- tive 37° C.	Total	Posi- tive 37° C.	Nega- tive 37° C.	Total	Posi- tive 37° C.	Nega- tive 37° C.	Total
	Percentage								
Positive.....	6.5	15.2	21.7	4.4	19.6	19.6	6.5	34.8	41.3
Negative.....	2.2	52.2	54.3	4.4	.....	4.4	6.5	52.2	58.7
* Total.....	8.7	67.4	76.1	4.4	19.6	23.9	13.0	87.0	100.0
	Number of specimens								
Positive.....	3	7	10	0	9	9	3	16	19
Negative.....	1	24	25	2	0	2	3	24	27
Total.....	4	31	35	2	9	11	6	40	46

<sup>1</sup> No culture was positive on potato dextrose agar plates incubated at 37° C.

It can readily be seen, from the data presented in table 1, that B. H. I. blood agar is the more efficient medium for the isolation of *Histoplasma capsulatum*, since 37 of 46, or 80.4 percent, of the positive cultures were obtained on this medium. Furthermore, it was much more efficient than potato dextrose agar, since only 19 of 46, or 41.3 percent, of the positive cultures were obtained on the latter medium.



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PLATE I

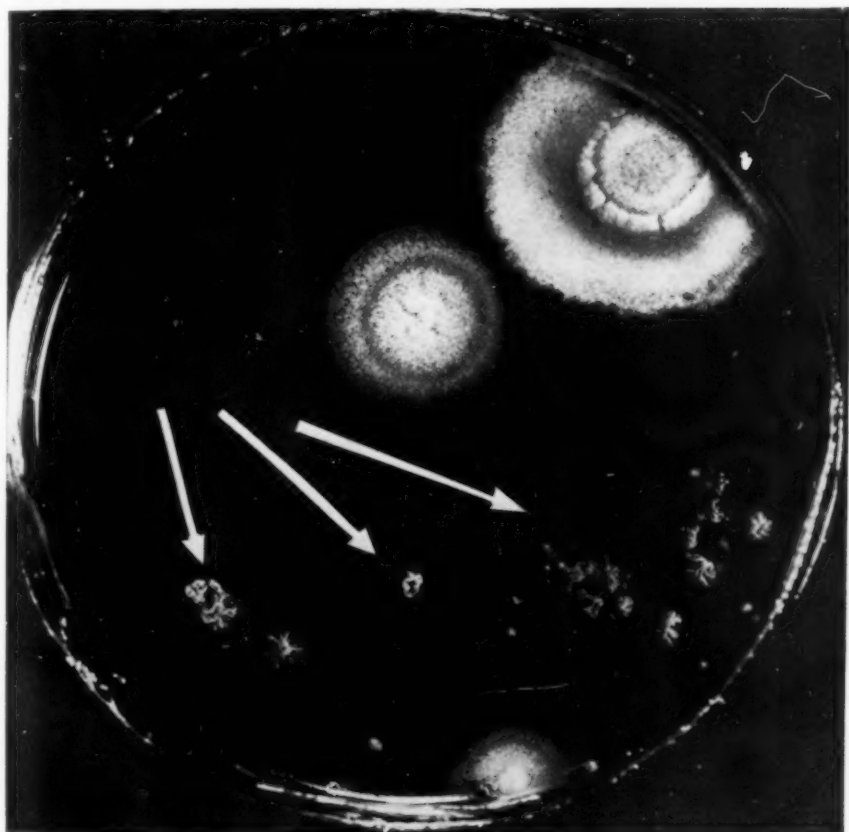


FIGURE 1.—Colonies of *Histoplasma capsulatum* on a blood agar plate incubated at room temperature.

PLATE 2

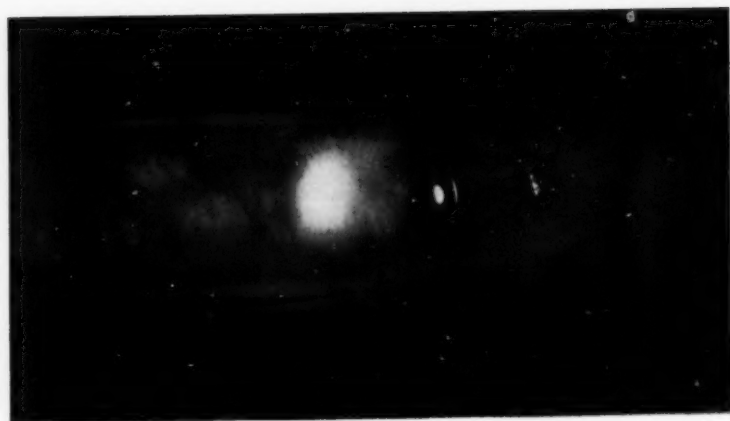


FIGURE 2.—*Histoplasma capsulatum* on a potato dextrose agar slant incubated at room temperature.

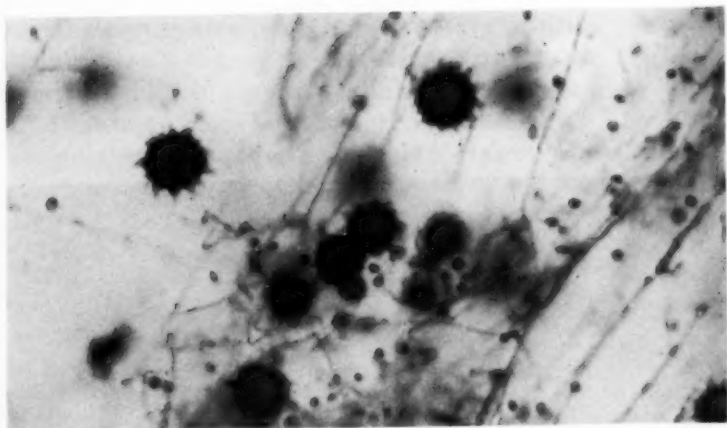


FIGURE 3.—Photomicrograph of growth from potato dextrose agar slant incubated at room temperature (864-X).

It is also evident, from the data presented in table 1, that the temperature at which the cultures were incubated was a very important factor in the isolation of *Histoplasma*. For example, while 35 of 46, or 76.1 percent, of the positive cultures were obtained on the blood agar plates incubated at room temperature, only 6 of 46, or 13.0 percent, were positive on this medium when the plates were incubated at 37° C. Similarly, while 19 of 46, or 41.3 percent, were positive on the potato dextrose agar plates incubated at room temperature, none was positive on plates of this medium incubated at 37° C.

It can also be seen, from the data presented in table 1, that 44 of 46, or 95.7 percent, of all positives were obtained by the use of the two media in plates incubated at room temperature.

While omission of any one of the procedures used, except for potato dextrose plates incubated at 37° C., would have decreased the number of positive cultures obtained, the omission of blood agar plates incubated at room temperature would have resulted in the greatest loss, as shown in table 1. For example, while the omission of the blood plates incubated at 37° C. would have decreased the number of positive cultures obtained by 2, or only 4.4 percent, and omission of potato dextrose agar plates incubated at room temperature would have decreased the total number of positives obtained by 9, or 19.6 percent, omission of the blood plates incubated at room temperature would have resulted in a loss of 24 positive cultures, or 52.2 percent of the total obtained, since this was the only procedure employed which gave a positive culture from these 24 animals. This confirms the experience of Heilman (10) who reported that he obtained the best results in the isolation of *Histoplasma capsulatum* from human cases by the use of blood agar plates incubated at approximately 30° C.

In addition to the higher efficiency of the blood agar, incubated at room temperature, for the isolation of *Histoplasma capsulatum*, it should be pointed out that colonies of this fungus, when grown under these conditions, are usually atypical in appearance. As described by De Monbreun (1), and as shown in figure 1 the colonies are usually moist, heaped, and somewhat cerebriform in appearance, varying in color from cream-colored to deep pink to reddish-brown. The growth from these colonies is usually devoid of spore forms, being composed entirely of mycelium. When subcultured on potato dextrose agar, and incubated at room temperature, however, they develop into the typical white, later cinnamon-brown, cottony type of growth, with abundant aerial mycelium (fig. 2) on which are produced abundant small and large chlamydospores with numerous large tuberculate chlamydospores characteristic of *Histoplasma capsulatum* (fig. 3) (1, 11).

## SUMMARY AND CONCLUSIONS

Positive cultures were obtained from the spleens of 46 guinea pigs experimentally infected with *Histoplasma capsulatum*. The media employed for isolation were brain-heart infusion blood agar and potato dextrose agar, with plates of each medium incubated at both room temperature and at 37° C. It was shown that:

1. Cultures on B. H. I. blood agar, incubated at room temperature, were the most efficient for the isolation of *Histoplasma capsulatum*.
2. Colonies of this fungus which develop on B. H. I. blood agar, incubated at room temperature, are moist, heaped, and cerebriform in appearance, and may develop a pink to reddish-brown pigmentation. Such colonies must be transferred to a medium such as potato dextrose agar, and incubated at room temperature, in order to obtain the characteristic tuberculate chlamydospores which allow positive identification.

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## STUDIES OF FUNGUS ANTIGENS

II. Preliminary Report on the Isolation of an Immunologically Active Polysaccharide from Histoplasmin<sup>1</sup>

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## INTRODUCTION

It has been shown (1, 2) that guinea pigs experimentally infected with either *Histoplasma capsulatum* or *Blastomyces dermatitidis* will react to histoplasmin. It was felt, therefore, that if the reacting principle could be isolated from histoplasmin it might be more specific and could be more accurately standardized. Accordingly, carbohydrates and protein fractions were extracted from histoplasmin by accepted chemical procedures. The following is a preliminary report of an immunologically active polysaccharide isolated from histoplasmin.

## MATERIALS AND METHODS

Several strains of both *Histoplasma capsulatum* and *Blastomyces dermatitidis* were used in these studies. Two lots of histoplasmin and one of blastomycin, designated as lots H-15, H-17, and B-7, respectively, were employed. These were prepared by a method similar to that used by Emmons et al. (1) as previously reported (2).

A polysaccharide fraction of histoplasmin was prepared from lot H-17 by a method suggested by Martin (3). In this technic, the proteins were removed by precipitation with glacial acetic acid. After addition of the acid the mixture was allowed to stand in the refrigerator overnight (5° C), and the precipitated proteins then removed by filtration through nitrogen-free filter paper and centrifugation.

After removal of the proteins, the polysaccharide was precipitated by the addition of four volumes of ethyl alcohol (95 percent) and the mixture was allowed to stand for 24 hours at 5° C. The precipitate was removed by centrifugation, and then dissolved in ten percent sodium acetate. After acidifying with acetic acid, and centrifugation to remove any free protein remaining, the polysaccharide was again precipitated with cold ethyl alcohol and allowed to stand at 5° C for 2 hours. This purification was repeated, and the resulting precipitate, removed by centrifugation, dissolved in distilled water. After a final precipitation with cold alcohol, the resulting precipitate was again removed by centrifugation and dried in vacuo over NaOH. The yield of polysaccharide was 0.2051 grams from 600 ml. of stock H-17 histoplasmin, or equal to 0.34 mg. per ml.

Testing solutions of the polysaccharide were prepared by dissolving the dried powder in sterile distilled water to make a final concentration

<sup>1</sup> From the Office of Field Studies, Tuberculosis Control Division.

of 10 mg./ml., with subsequent sterilization by filtration through a Seitz filter.

Examination of this solution for the presence of free protein, using trichloroacetic acid, Millon's reagent, ammonium sulfate and xanthoproteic and biuret reactions indicated that no free proteins were present. This solution contained 0.464 mg./ml. nitrogen. Preliminary tests for reducing sugars using Benedict's reagent and the Shaffer-Hartman and Somogyi methods (4, 5) indicated that such sugars were absent. However, when a small sample was hydrolysed with normal hydrochloric acid and subsequently neutralized and tested with copper sulfate (4), reducing sugars were found to be present.

As a control for the skin test studies, a small amount of the stock solution of lot H-17 histoplasmin was dried in vacuo over NaOH, and the residue diluted with distilled water to make a final concentration of 10 mg. per ml. The yield of total solid was 1.2799 grams from 40 ml. of stock H-17, or equal to 32 mg. per ml.

Each of 25 albino guinea pigs were tested by the intradermal injection of a skin test dose (0.1 cc.) of a 1-100 dilution of lot H-15 histoplasmin and 1 mg. per ml. of the polysaccharide fraction of lot H-17. Fifteen of these normal animals were also tested with the same concentration of lot H-17 (1 mg./ml.). None of these normal animals reacted to either antigen in this dilution. Thirty-four additional normal albino guinea pigs were then tested similarly with lot H-15. Since none of these normal animals reacted to this dilution, they were inoculated by the intraperitoneal injection of a saline suspension of the yeast phase of *H. capsulatum*. Five to seven weeks after inoculation, each of the guinea pigs was tested with several dilutions of lots H-15 and H-17 histoplasmin and the polysaccharide fraction prepared from lot H-17. Reactions of guinea pigs to the polysaccharide fraction, as previously reported for histoplasmin (1, 2) reach their height at 24 hours, and may disappear within 48 hours. Only those tests which showed 5 mm. or more of induration were considered reactors.

1. *Titration of polysaccharide fraction of histoplasmin.*—The results of testing guinea pigs experimentally inoculated with *Histoplasma capsulatum* with the polysaccharide isolated from lot H-17 histoplasmin are shown in table 1.

It is evident, from the data presented in table 1 that guinea pigs experimentally inoculated with *H. capsulatum* will react to the polysaccharide fraction of histoplasmin.

It is also evident, from the data presented in table 1, that, as previously shown for various lots of histoplasmin (2) the percentage of experimentally inoculated animals which will react to the polysaccharide fraction of histoplasmin, and the size of the reaction, is dependent upon the dosage employed. For example, while 31 of



33, or 93.9 percent, reacted to a dosage of 0.1 mg., 26 of 33, or 78.8 percent, reacted to 0.01 mg., and 15 of 33, or 45.5 percent, to 0.001 mg.

TABLE 1.—Results of testing with various dilutions of lots H-15 and H-17 histoplasmin and the polysaccharide fraction of lot H-17 in guinea pigs experimentally inoculated with *Histoplasma capsulatum*

Item	Histoplasmin				Polysaccharide fraction of histoplasmin		
	Lot H-15 dilution		Lot H-17 dilution of 1 ml. containing—		Lot H-17 dilution of 1 ml. containing—		
	1-100	1-1000	1.0 mg. solid	0.1 mg. solid	1.0 mg.	0.1 mg.	0.01 mg.
Number of animals tested.....	34	33	33	33	33	33	33
Number of reactors.....	29	22	27	21	31	26	15
Percentage of reactors.....	85.3	66.7	81.8	63.6	93.9	78.8	45.5
Average diameter of reaction <sup>1</sup> .....	12.5	7.2	8.6	6.9	13.3	9.1	6.2

<sup>1</sup> Induration in millimeters.

Therefore, as for histoplasmin (2), it would seem to be very important to determine the critical titer or dosage of any fraction of histoplasmin to be employed to determine sensitization of an individual by *Histoplasma*.

It has been suggested (2) that for practical purposes the critical titer or dosage of any antigen to be used as a skin testing antigen be the minimal amount of that antigen which would detect sensitivity in approximately 80-90 percent of such a group of animals. On this basis, it would seem, from the data presented in table 1, that 0.1 to 0.01 mg. would be for practical use, the critical dosage of the polysaccharide fraction of histoplasmin. However, from a comparison of the data presented in table 1 and data previously presented (2) it is evident that this group of animals (table 1) were not at the height of their sensitivity level at the time they were tested since a dilution of lot H-15 histoplasmin gave a reaction in only 22 of 33, or 66.7 percent of the animals sensitized with *Histoplasma*, whereas it has previously been shown (2) that this dilution of lot H-15 should detect 85 to 90 percent of such a group of animals. From this comparison, therefore, it would seem that for practical use the critical titer of the polysaccharide fraction of histoplasmin would probably be a dosage of between 0.01 and 0.001 mg., even though in the group of animals reported in table 1 these dosages gave reactions in only 78.8 and 45.5 percent of the animals tested.

2. Cross-reactions of the polysaccharide fraction of histoplasmin.—In addition to the studies reported above in which the polysaccharide fraction of histoplasmin was tested on guinea pigs experimentally inoculated with *Histoplasma capsulatum*, this fraction was also tested



on guinea pigs experimentally inoculated with *Blastomyces dermatitidis*, using lot B-7 blastomycin as a control. The results of these tests are summarized in table 2.

TABLE 2.—Results of testing with various dilutions of lot B-7 blastomycin and the polysaccharide fraction of lot H-17 histoplasmin in guinea pigs experimentally inoculated with *Blastomyces dermatitidis*

Item	Blastomycin—Lot B-7 dilution		Polysaccharide fraction of histoplasmin—Lot H-17 dilution of 1 ml. containing—		
	1-100	1-1000	1.0 mg.	0.1 mg.	0.01 mg.
Number of animals tested.....	28	24	27	27	27
Number of reactors.....	27	12	13	7	1
Percentage of reactors.....	96.4	50.0	48.1	25.9	3.7
Average diameter of reactions <sup>1</sup> .....	10.7	6.1	7.7	5.9	6.0

<sup>1</sup> Induration in millimeters.

It can be seen, from the data presented in table 2, that guinea pigs experimentally inoculated with *B. dermatitidis* will react to the polysaccharide fraction of histoplasmin. As previously noted for histoplasmin (2), however, and for animals inoculated with *Histoplasma* (table 1), the number of animals which react, and the size of the reaction, is dependent upon the dosage employed (table 2).

As was noted above for the animals sensitized with *Histoplasma* (table 1), the animals reported in table 2 were also apparently not at the peak of their sensitivity level; as has been shown previously (2), at the peak of sensitivity a 1-1000 dilution of lot B-7 blastomycin should give reactions in at least 85 to 90 percent of animals sensitized by *B. dermatitidis*. It is possible, therefore, that had they been at the peak, the number which reacted to the polysaccharide fraction of histoplasmin might have been higher than the numbers shown in table 2. However, it would seem unlikely, in view of the percentage of reactors obtained, that this percentage of cross-reactions would be very high if the critical titer of the polysaccharide fraction is employed as a skin test antigen.

#### DISCUSSION

It has been shown above (table 1) that almost all guinea pigs experimentally inoculated with *Histoplasma capsulatum* will react to the polysaccharide fraction isolated from histoplasmin. It has also been shown (table 1) that the number and percentage of such animals which reacted to this fraction were equal to, or greater than, the number and percentage which reacted to the same concentration of the residue from the stock solution of the same lot of histoplasmin. For example, 26 of 33, or 78.8 percent, reacted to 0.01 mg. of the polysaccharide while only 21 of 33, or 63.6 percent, reacted to the same dosage of the residue of the stock solution (table 1).

The polysaccharide yield was only one percent of the total solids in H-17. However, since the percentage of the total solid in stock H-17 which is immunologically active is unknown, the percentage yield of the reacting principle cannot be determined. Therefore, although it is not possible to state that none of the active principle was lost during the process of purification, it would seem that at least some of the active principle of histoplasmin is polysaccharide in nature.

Further studies on the activity and specificity of both polysaccharide and protein fractions of histoplasmin are in progress.

#### SUMMARY

A polysaccharide fraction shown to be free from protein by a variety of qualitative tests has been isolated from one lot of histoplasmin. This antigen has been tested on guinea pigs experimentally inoculated with *Histoplasma capsulatum* and *Blastomyces dermatitidis*, using histoplasmin and blastomycin, respectively, as controls.

It has been demonstrated that:

1. This polysaccharide fraction of histoplasmin will give reactions in guinea pigs experimentally inoculated with either *Histoplasma capsulatum* or *Blastomyces dermatitidis*.
2. The number of animals inoculated with either fungus which will react to this antigen is dependent upon the dosage employed.
3. It is suggested that the active principle of histoplasmin is, at least in part, polysaccharide in nature, and that if the critical titer of this antigen is determined, it will be found to be relatively specific for animals infected with *Histoplasma capsulatum*.

#### ACKNOWLEDGMENT

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## ON THE VERIFICATION OF THE DIAGNOSIS OF TUBERCULOSIS

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The various campaigns now in progress for the early diagnosis of tuberculosis through X-ray surveys have brought about a gradual change in the type of case admitted for sanatorium care. Ten or fifteen years ago, a patient usually consulted his physician because of symptoms, and may have been referred to the sanatorium following an X-ray. At present, the sequence of diagnostic procedures is likely to be reversed: an increasingly large proportion of patients is admitted to tuberculosis sanatoria because of X-ray findings alone. Under such conditions, there will be, necessarily, an increased proportion of patients who, after exhaustive study, are found not to have pulmonary tuberculosis at all, for the expert roentgenologist will be the first to admit that the diagnosis of tuberculosis from the X-ray alone is by no means infallible (1). So, increasingly, the clinical staff of the sanatorium is forced to fall back on the laboratory for some definite confirmation of the roentgenographic diagnosis.

The importance of obtaining laboratory evidence as quickly as possible to decide whether the afflicted person actually has tuberculosis may be emphasized from two points of view; that of the patient, and that of the public health program. From the point of view of the patient, the diagnosis of tuberculosis carries with it a certain amount of mental shock. Obviously this should not be inflicted on any person unnecessarily, nor should the patient who does not have tuberculosis be exposed to the hazard of tuberculosis contact in the wards of a sanatorium. From the point of view of the public health program, the entire early diagnosis campaign will be discredited unless a swift and accurate diagnosis of each patient's condition is made (2). From the same point of view, the very limited sanatorium facilities for the tuberculous should not be tied up unnecessarily by patients who do not actually require sanatorium care (3).

### METHOD

As a partial answer to the need for rapid laboratory confirmation or negation of the X-ray diagnosis of tuberculosis, some sanatoria have again brought into active use a long-neglected procedure—the routine tuberculin testing of patients. At the Wm. H. Maybury Sanatorium, a tuberculin test is given to every newly admitted adult patient. One-tenth ml. of a 1:10,000 dilution of Old Tuberculin (0.01 mg.)<sup>2</sup> is

<sup>1</sup> From the Wm. H. Maybury Sanatorium (Detroit Municipal Tuberculosis Sanatorium) Northville, Michigan.

<sup>2</sup> All of the tuberculin used in this study was kindly furnished by Parke, Davis and Company of Detroit.

injected intracutaneously by the medical technologist at the same time that blood is taken for the admission blood count. Reactions are read at the end of 48 hours, and an area of induration or edema of 5 mm. or more in diameter is considered a positive reaction. Patients who are nonreactors to the first dilution are retested at 48-hour intervals with tuberculin ten times as concentrated, and with successively stronger dilutions until the final dilution 1:10 OT (10 mg.) is reached.

Testing with the successively more concentrated tuberculin makes it possible to assign each patient to a "sensitivity level" or "sensitivity group," depending upon the dilution of tuberculin to which he reacts (4). Thus, all patients who show the usual reaction to the 1:10,000 dilution of tuberculin are included in the "10,000" sensitivity group. Those patients who fail to react to 1:10,000 OT, but who show a positive reaction to 1:1,000 OT, are assigned to the "1,000" group, and a similar procedure is followed for the other dilutions. Patients who fail to react to 1:10 OT are described in the present study as *anergic* and are placed in the "0" sensitivity group.

An additional sensitivity group, the "100,000" group, has been created for those patients who show the most marked hypersensitivity to tuberculin. Patients with a reaction to 1:10,000 OT averaging 35 mm. or more in diameter are retested with the 1:100,000 dilution. In nearly every instance, they have been found to give a positive reaction to this very minute quantity of tuberculin and have been placed in the "100,000" sensitivity group.

In addition to the tuberculin and other routine tests, every effort is made to demonstrate tubercle bacilli in the sputum of the patient. If stained smears fail to reveal acid-fast bacilli, repeated sputum cultures are made, to be followed in some instances by cultures of the gastric contents.

#### SENSITIVITY GROUPS

During the 2-year period, January 1, 1945 to January 1, 1947, 1,376 patients, newly admitted to the Wm. H. Maybury Sanatorium, were adequately studied with regard to both sputum and tuberculin test.<sup>3</sup> These individuals varied in age from 13 to 83 years, were of both sexes, and included approximately 15 percent colored patients. In every case a tentative diagnosis of tuberculosis had been made, by X-ray or other means, before the patient was admitted to Maybury. Table 1 gives the classification of the patients according to tuberculin sensitivity and sputum findings.

<sup>3</sup> During these 2 years, 1,715 patients were admitted to the Sanatorium. Of these, 297 were admitted to the children's unit and are excluded from the present study for that reason. Thirteen adults are excluded either because they left the Sanatorium against advice or were discharged before their series of tuberculin tests had been completed. Twenty-nine adults are excluded because death occurred before their tuberculin tests had been completed. Twenty-eight of these latter were proved tuberculous by either sputum findings or postmortem examination. The one nontuberculous death was due to carcinoma of the lungs.

It will be seen from table 1 that in more than 50 percent of the cases, the sputum was found to contain tubercle bacilli following the examination of direct or concentrated smears. This information was available 4 days after the patient's admission to the sanatorium. In another 22.9 percent of the cases, the clinician had to wait 3 to 8 weeks, or longer, for a report from cultures of the sputum of gastric contents. More important still, in 25.6 percent of the cases, direct bacteriologic proof of infection with tubercle bacilli was not found. In contrast to the bacteriologic findings, the totals for the sensitivity groups indicate that the great majority of the patients fall in the first three groups. The total for these 3 groups is 1,302, or 94.7 percent of the grand total of 1,376 patients; the results from these first 2 tuberculin tests, also, were available to the clinician on the fourth day after the admission of the patient.

TABLE 1.—*Classification of 1,376 patients according to tuberculin sensitivity and sputum findings*

Tuberculin sensitivity level <sup>1</sup> group	Total		Sputum findings in patients					
			Smear positive		Smear negative <sup>2</sup> culture positive		Smear and culture negative	
	Number	Percent	Number	Percent	Number	Percent	Number	Percent
Total	1,376	100.0	708	51.5	315	22.9	353	25.6
100,000	53	100.0	15	28.2	13	24.6	25	47.2
10,000	1,072	100.0	550	51.3	270	25.2	252	23.5
1,000	177	100.0	111	62.7	26	14.7	40	22.6
100	24	100.0	16	66.7	3	12.5	5	20.8
10	14	100.0	7	50.0	1	7.1	6	42.9
0	36	100.0	29	25.0	2	5.6	25	69.4

<sup>1</sup> Patients are tested with varying doses of OT; assignment to group 100,000, 10,000, 1,000, 100, or 10 indicates reaction to the specified strength of OT, while group 0 designates anergic patients failing to react to the dosage 1:10 OT.

<sup>2</sup> The number of cases in this particular group would have been materially increased had it been possible to include the 28 tuberculous patients who died before their tuberculin tests had been completed. See footnote 3, on page 185.

<sup>3</sup> The column "Smear negative-culture positive" includes 14 cases recorded as positive because of the culture of gastric contents, the presence of tubercle bacilli in the stomach being the result, presumably, of swallowed sputum.

While a high level of sensitivity to tuberculin is not a response upon which to base a clinical diagnosis of tuberculosis, experts in the field agree that skin sensitivity to the 1:1000 dilution of tuberculin, or to lesser amounts, at least indicates that the patient at some previous time has been infected with tubercle bacilli. Thus, on the fourth day after the patient's admission, the tuberculin test gave presumptive evidence of tuberculous infection in 94.7 percent of the patients while examination of the sputum, after 4 days, yielded positive results in only 51.5 percent of the cases.

A brief scanning of table 1 reveals a considerable variation in sensitivity among the relative number of patients who were found positive on smear, positive on culture alone, or who were negative following both smear and culture examinations. These variations

are shown diagrammatically in figure 1. It will be seen that the proportion of patients for whom tubercle bacilli are shown in smears of the sputum increases with the decrease in sensitivity until a maximum of 66.7 percent is reached at the "100" sensitivity level. On the other hand, the proportion of patients for whom both smear and culture examinations were negative, changes in practically inverse ratio; the maximum for this group is found at the "0" sensitivity level. The explanation for these changes is to be found in the fact that the anergic or partially anergic patients fall into two well defined clinical

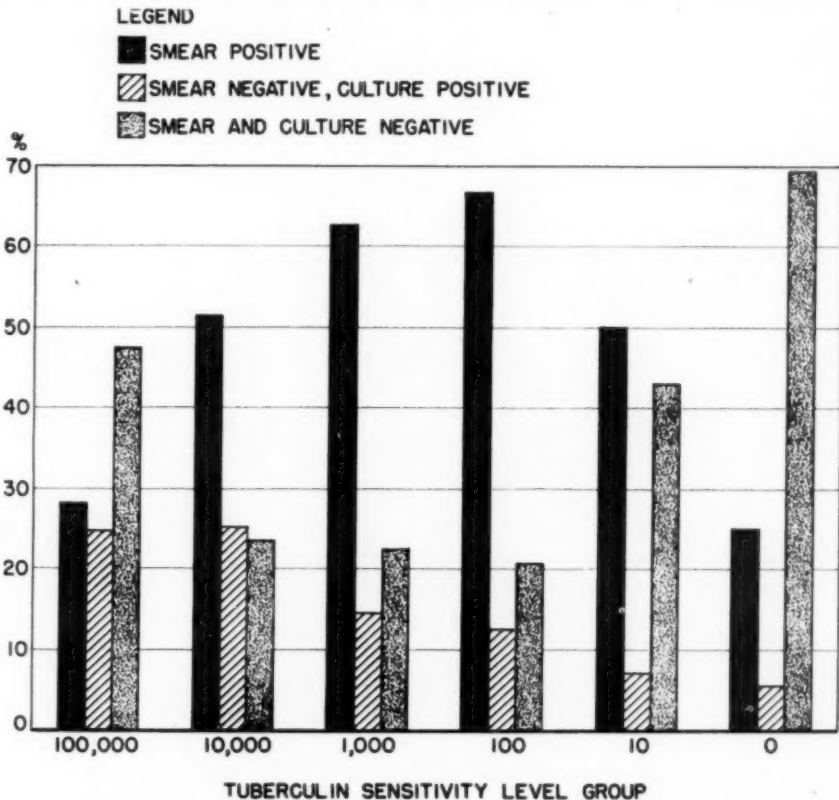


Figure 1.—Tuberculin sensitivity as related to sputum findings in newly admitted sanatorium patients.

groups: those who are critically ill with tuberculosis and who are anergic for that reason, and those who do not have tuberculosis at all (5). For patients who are anergic because of their extensive disease, tubercle bacilli will be demonstrated in direct smears of the sputum in nearly every case; (5, 6), while in the nontuberculous patients it is impossible to demonstrate tubercle bacilli no matter how many cultures of the sputum or gastric contents are made.

As mentioned above, the patient with active tuberculosis who is anergic is always critically ill. In our experience these cases have always had a fatal outcome within 6 weeks of their admission to the



sanatorium. On the other hand, the nontuberculous anergic patients usually have no symptoms at all, in spite of X-ray pictures which may look alarming. Most of these patients are discharged from the sanatorium with the diagnosis of sarcoid disease, bronchiectasis, atypical pneumonia, tracheobronchial lymphadenitis, or one of the fungus infections.

#### DISCUSSION

The nationwide campaigns now being fostered for the early diagnosis of tuberculosis are having two effects: the desirable one of causing the discovery of more cases of minimal tuberculosis, and the undesirable one of causing occasional patients who do not have tuberculosis to be sent to tuberculosis sanatoria. The dangers of this latter development to both the patient and the public health program have been pointed out, as well as the necessity for determining as soon as possible, in the case of each newly-admitted patient, whether he should remain in the sanatorium environment.

Routine tuberculin tests, started on the day a patient is admitted to the sanatorium, can be of considerable aid in determining whether the patient actually has tuberculosis. Such tests will, on the fourth day after admission, give presumptive evidence of tuberculous infection in approximately 95 percent of new admissions; the examination of sputum smears for tubercle bacilli will yield positive results in only a little over 50 percent of the same patients.

It is evident that the 95 percent of newly admitted patients who react to 0.1 mg or less of tuberculin will include an occasional patient whose major pulmonary disease is something other than tuberculosis—carcinoma or fungus infection for example (7, 8, 9). However, the evidence of a previous infection with tubercle bacilli given by this level of tuberculin sensitivity justifies keeping the patient in the sanatorium for a more leisurely determination of the diagnostic problem involved.

In the approximately 5 percent of patients who *fail* to react to the 1:1000 dilution of tuberculin it is important to continue the tests to determine as accurately as possible, the level of tuberculin sensitivity of each patient. The present study indicates that with the decrease in sensitivity to tuberculin there is an increasing tendency for the patients to fall into two groups: those who are critically ill with tuberculosis and who are likely to show tubercle bacilli in direct or concentrated sputum smears, and those in relatively good condition who do not have tuberculosis at all. Determining to which of these two groups the anergic patient belongs is of the greatest importance, for in one case he is definitely in need of isolation in an institution for the tuberculous, and in the other, he should be removed from such an institution as quickly as possible.

With the additional help of a good history and physical examination, differentiation between the tuberculous and the nontuberculous



anergic patient is usually simple; in most cases it can be done merely by determining whether or not the patient is acutely ill. Anergic patients in good physical condition, whose initial studies fail to show tubercle bacilli in smears of the sputum, should be discharged without waiting for the results of sputum cultures, for in our experience such cultures have been either completely negative or have yielded only acid-fast saprophytes. Three times during the past 4 years the laboratory has reported sputum cultures positive for tubercle bacilli in anergic patients who were not clinically ill. In each instance, because the patient was anergic the culture was inoculated immediately into a guinea pig. Every one of the cultures failed to produce tuberculosis in the animal. In each instance these cultures had been "read" by individuals with long experience in tuberculosis laboratory work. (Smears of the cultures had not been made.) Thus, in these cases the quantitative tuberculin test served as an effective check on the most definitive report given by the laboratory—that regarding the presence or absence of tubercle bacilli in the sputum (5). It should be re-emphasized that virulent tubercle bacilli are not found in the sputum of an anergic patient unless he is critically ill.

#### SUMMARY

Of 1,376 patients admitted to a sanatorium because of roentgenographic or other findings suggestive of tuberculosis, 51.5 percent revealed tubercle bacilli in direct or concentrated smears of the sputum; 94.7 percent were found to react to 1:1000 OT or to less concentrated dilutions.

The advantages of determining accurately the level of tuberculin sensitivity of each newly admitted patient are indicated.

By combining an accurate sputum study and determination of tuberculin sensitivity with an estimation of the condition of the patient, the clinician is better able to assess the roentgenographic findings in suspected cases of tuberculosis.

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## MECHANISM OF IMMUNITY AND ALLERGY IN TUBERCULOSIS<sup>1 2</sup>

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### Conclusions

1. Natural resistance to TB varies between widely separated extremes in man as well as in animals, determining the course followed by tuberculosis infection.

2. Tuberculosis infection is accompanied by heightened resistance to subsequent contagion. Acquired immunity to tuberculosis does not fit into the framework of typical immunity to infection, but differs from it in several peculiarities.

3. Like natural immunity, acquired immunity is characterized by lung stability in the organism and cessation of multiplication of tubercle bacilli. Parallel with this, there are the processes of destruction, elimination, and calcification.

4. Tuberculosis infection is accompanied by an increased sensitivity to the various components of the tubercle bacillus (specific allergy), as well as to nonspecific antigens (hetero-allergy).

5. Tuberculosis allergy does not represent an anaphylactic phenomenon but rather a phenomenon of intoxication. The central nervous system is especially sensitive to products of the tubercle bacillus.

6. Tuberculosis immunity and allergy do not appear identically, nor do they invariably follow a parallel course of development. They may reveal themselves simultaneously in the organism, but they are easily dissociated experimentally.

7. The dynamics of the development of allergy and immunity and their relations differ in the various types of tuberculous illness. It is possible to establish some regularities in the development of tuberculous processes in connection with immuno-biological modifications.

8. The study of the mechanism of immuno-biological factors in tuberculosis will make it possible to influence them for therapeutic and prophylactic purposes.

<sup>1</sup> Published in "Problemy Tuberkuleza, 1946, No. 2: 3-14. Ministry of Public Health, Moscow, U. S. S. R.

<sup>2</sup> Translated from Russian by the Office of the Chief, Tuberculosis Control Division, U. S. Public Health Service.

# INCIDENCE OF DISEASE

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*No health department, State or local, can effectively prevent or control disease without knowledge of when, where, and under what conditions cases are occurring*

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## UNITED STATES

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### REPORTS FROM STATES FOR WEEK ENDED JANUARY 17, 1948

#### Summary

The number of reported cases of influenza increased slightly during the week—from 10,335 cases last week to 10,360 for the current week. More than 90 percent of the cases were reported in 8 States in the southern and southwestern areas, which were the only States reporting more than 200 cases. These States are as follows (last week's figures in parentheses): Texas 4,509 (4,712), Arizona 1,039 (849), California 1,023 (1,272) South Carolina 880 (916), Virginia 868 (849), Oklahoma 442 (124), Arkansas 439 (452) and Alabama 265 (277).

The outbreaks in which the clinical symptoms have been reported have presented a varied clinical picture in which gastro-intestinal symptoms have predominated and including sore throat and various other upper-respiratory infections. The disease has apparently been reported generally as mild, with sudden onset, of short duration, and relatively low temperatures. The great variation in symptoms indicates that epidemic nausea, vomiting and diarrhea and the common cold are also present in some of the reported outbreaks.

The incidence of poliomyelitis has dropped to about the normal seasonal level—40 cases were reported currently, as compared with 41 for the preceding week, 91 for the corresponding week in 1947 and a 5-year (1943-47) median of 46 for the week.

One case of smallpox each was reported in Missouri, Idaho, Colorado and Arizona, and 1 case of anthrax each in Massachusetts and Pennsylvania. Diphtheria, meningococcus meningitis, scarlet fever and typhoid fever are below previous low figures, while the incidence of measles is above that for any prior year since 1944 and whooping cough higher than for any other year since 1943.

During the current week 10,150 deaths, all causes, were reported in 93 large cities in the United States, as compared with 11,313 for the preceding week, 9,960 for the corresponding week of 1947, and 10,401 for the same week in 1946. The cumulative total for the first 3 weeks this year is 31,881, as compared with 30,807 for the same period last year.

*Telegraphic morbidity reports from State health officers for the week ended Jan. 17, 1948, and comparison with corresponding week of 1947 and 5-year median*

In these tables a zero indicates a definite report, while leaders imply that, although none was reported, cases may have occurred.

Division and State	Diphtheria			Influenza			Measles			Meningitis, meningococcus		
	Week ended—		Median 1943-47	Week ended—		Median 1943-47	Week ended—		Median 1943-47	Week ended—		Median 1943-47
	Jan. 17, 1948	Jan. 11, 1947		Jan. 17, 1948	Jan. 11, 1947		Jan. 17, 1948	Jan. 11, 1947		Jan. 17, 1948	Jan. 11, 1947	
NEW ENGLAND												
Maine.....	1	6	4				1	292	16	0	1	1
New Hampshire.....	0	0	0				1	22	12	0	0	0
Vermont.....	0	1	0				19	117	8	0	1	0
Massachusetts.....	1	19	4				226	443	358	3	1	8
Rhode Island.....	0	2	2			9		25	9	0	0	0
Connecticut.....	0	0	1		1	4	9	124	61	1	3	4
MIDDLE ATLANTIC												
New York.....	17	36	16	13	17	22	460	246	852	9	9	25
New Jersey.....	3	5	5	8	6	26	747	76	76	4	4	11
Pennsylvania.....	8	13	16	(2)	23	25	348	1,221	776	5	4	16
EAST NORTH CENTRAL												
Ohio.....	5	17	15	6	8	14	648	286	61	0	1	14
Indiana.....	6	10	11	23	19	19	203	11	46	0	3	0
Illinois.....	0	4	5	2	2	11	1,114	17	176	3	6	9
Michigan.....	4	13	7	2	4	5	837	23	135	1	0	9
Wisconsin.....	0	0	1	51	20	147	217	147	147	1	3	4
WEST NORTH CENTRAL												
Minnesota.....	0	12	6			2	320	12	12	0	0	2
Iowa.....	1	1	6				111	31	31	0	2	1
Missouri.....	4	6	5	6	9	10	40	5	43	3	6	6
North Dakota.....	0	1	1	13	37	46	28	2	5	1	0	0
South Dakota.....	0	5	2				11	2	19	0	1	1
Nebraska.....	0	1	6	16	25	31	11	4	20	0	0	1
Kansas.....	5	1	4	3	86	86	10	4	65	2	2	4
SOUTH ATLANTIC												
Delaware.....	1	0	0				13	1	2	0	0	0
Maryland.....	22	14	12	6	5	22	23	33	22	1	2	7
District of Columbia.....	0	0	0	1	3	3	49	19	13	2	0	1
Virginia.....	8	14	11	868	504	504	50	145	145	2	8	10
West Virginia.....	2	2	5	159	98	98	230		15	3	1	1
North Carolina.....	21	10	15				1	155	36	3	1	3
South Carolina.....	5	9	6	880	774	854	7	70	70	1	3	3
Georgia.....	5	3	9	77	14	157	25	60	21	1	0	2
Florida.....	2	5	5	9	18	4	32	9	17	0	6	6
EAST SOUTH CENTRAL												
Kentucky.....	4	11	8		2	47	30	2	152	4	6	6
Tennessee.....	6	13	9	110	31	63	40	4	68	3	2	6
Alabama.....	11	4	5	265	51	265	14	21	19	2	1	7
Mississippi.....	5	6	9	15			37			0	3	5
WEST SOUTH CENTRAL												
Arkansas.....	2	8	9	439	144	158	44	29	29	1	0	3
Louisiana.....	10	8	8	180	26	26	14	1	22	5	4	4
Oklahoma.....	7	2	8	442	97	189	16	8	14	2	1	2
Texas.....	19	32	35	4,509	2,397	2,397	640	59	87	8	1	14
MOUNTAIN												
Montana.....	3	2	1	19	21	21	81	188	26	0	0	1
Idaho.....	1	0	0	52	23	2	2	6	12	0	0	0
Wyoming.....	0	1	0		12	28	11	1	8	2	0	0
Colorado.....	6	6	7	69	50	50	25	14	78	0	1	2
New Mexico.....	1	3	2	1	4	4	4	41	2	1	1	1
Arizona.....	5	2	2	1,039	181	181	4	77	7	0	1	1
Utah.....	4	0	0	2	12	12	7	2	21	0	0	1
Nevada.....	0	0	0				1		3	1	1	1
PACIFIC												
Washington.....	0	5	5	12			111	32	102	1	1	9
Oregon.....	1	4	4	50	16	27	24	42	58	0	0	2
California.....	13	23	29	1,023	7	68	476	86	258	16	9	23
Total.....	219	340	340	10,360	4,728	4,728	7,372	4,215	5,314	92	100	262
2 weeks.....	477	706	706	20,695	8,393	8,719	14,608	7,210	8,083	177	183	489
Seasonal low week <sup>1</sup> .....	(27th) July 5-11			(30th) July 26-Aug. 1			(35th) Aug. 30-Sept. 5			(37th) Sept. 13-19		
Total since low.....	6,835	8,272	9,149	64,253	41,368	41,368	49,554	30,097	34,207	959	1,155	1,957

<sup>1</sup> New York City only.

<sup>2</sup> Philadelphia only.

<sup>3</sup> Period ended earlier than Saturday.

<sup>4</sup> Dates between which the approximate low week ends. The specific date will vary from year to year.

Telegraphic morbidity reports from State health officers for the week ended Jan. 17, 1948, and comparison with corresponding week of 1947 and 5-year median—Con.

Division and State	Polio myelitis			Scarlet fever			Smallpox			Typhoid and para-typhoid fever		
	Week ended—		Med-ian 1943-47	Week ended—		Med-ian 1943-47	Week ended—		Med-ian 1943-47	Week ended—		Med-ian 1943-47
	Jan. 17, 1948	Jan. 11, 1947		Jan. 17, 1948	Jan. 11, 1947		Jan. 17, 1948	Jan. 11, 1947		Jan. 17, 1948 <sup>1</sup>	Jan. 11, 1947	
NEW ENGLAND												
Maine.....	0	1	0	14	27	27	0	0	0	0	1	0
New Hampshire.....	0	0	0	2	3	9	0	0	0	0	0	0
Vermont.....	0	0	0	5	4	4	0	0	0	1	1	0
Massachusetts.....	0	1	1	120	148	241	0	0	0	2	4	0
Rhode Island.....	0	0	0	9	5	14	0	0	0	0	0	0
Connecticut.....	0	1	0	43	32	57	0	0	0	0	0	0
MIDDLE ATLANTIC												
New York.....	4	4	4	210	286	399	0	0	0	2	3	3
New Jersey.....	0	0	0	62	102	103	0	0	0	0	0	1
Pennsylvania.....	4	0	0	204	159	272	0	0	0	3	2	2
EAST NORTH CENTRAL												
Ohio.....	0	0	1	237	309	265	0	1	0	0	0	1
Indiana.....	1	7	1	56	82	83	0	0	0	0	1	1
Illinois.....	1	2	1	122	127	223	0	0	0	1	3	1
Michigan <sup>2</sup> .....	1	2	2	136	119	118	0	0	0	0	0	0
Wisconsin.....	0	0	0	71	88	141	0	0	0	0	0	0
WEST NORTH CENTRAL												
Minnesota.....	1	1	1	40	35	63	0	0	0	0	0	0
Iowa.....	0	2	1	40	33	63	0	0	0	0	1	0
Missouri.....	0	2	1	37	34	76	1	0	0	0	1	1
North Dakota.....	0	0	0	7	4	12	0	0	0	0	0	0
South Dakota.....	0	1	0	8	4	23	0	0	0	0	0	0
Nebraska.....	0	1	0	40	38	33	0	0	0	0	0	0
Kansas.....	0	5	0	25	42	64	0	0	1	0	1	0
SOUTH ATLANTIC												
Delaware.....	0	0	0	9	5	5	0	0	0	0	0	0
Maryland <sup>3</sup> .....	0	0	0	27	37	66	0	0	0	0	0	0
District of Columbia.....	0	0	0	10	16	25	0	0	0	0	0	0
Virginia.....	0	0	0	27	52	53	0	0	0	2	1	1
West Virginia.....	1	1	0	14	24	53	0	0	0	1	0	0
North Carolina.....	2	4	1	20	19	52	0	0	0	0	1	1
South Carolina.....	0	0	0	4	19	17	0	0	0	0	0	1
Georgia.....	0	1	0	11	9	13	0	1	0	0	1	1
Florida.....	1	3	1	11	18	8	0	0	0	1	0	1
EAST SOUTH CENTRAL												
Kentucky.....	1	0	0	42	41	43	0	0	0	2	0	1
Tennessee.....	0	4	1	40	30	57	0	1	0	4	5	2
Alabama.....	0	1	1	12	14	15	0	0	1	0	1	0
Mississippi <sup>2</sup> .....	0	3	0	11	10	13	0	0	0	1	4	1
WEST SOUTH CENTRAL												
Arkansas.....	0	2	1	5	3	8	0	2	0	0	0	0
Louisiana.....	1	2	0	7	5	10	0	0	0	2	3	4
Oklahoma.....	0	0	0	42	6	18	0	0	1	0	0	1
Texas.....	0	7	1	40	41	62	0	0	0	3	5	5
MOUNTAIN												
Montana.....	0	0	0	23	3	21	0	0	0	0	0	0
Idaho.....	0	7	0	7	16	16	1	0	0	0	0	0
Wyoming.....	0	1	1	1	3	9	0	0	0	0	0	0
Colorado.....	1	2	1	16	45	45	1	0	0	0	0	0
New Mexico.....	0	0	0	8	14	13	0	0	0	0	0	0
Arizona.....	0	0	1	7	5	11	1	0	0	0	0	1
Utah <sup>3</sup> .....	2	0	0	20	17	67	0	0	0	0	0	0
Nevada.....	0	0	0	3	0	0	0	0	0	0	0	0
PACIFIC												
Washington.....	10	2	1	80	42	42	0	0	0	0	2	1
Oregon.....	4	2	1	26	23	23	0	0	0	1	0	0
California.....	5	19	7	80	138	195	0	0	0	0	2	2
Total.....	40	91	46	2,091	2,336	3,637	4	5	9	26	43	43
2 weeks.....	81	170	84	4,088	4,416	7,094	8	8	21	71	81	81
Seasonal low week <sup>4</sup> .....	(11th) Mar. 15-21			(32nd) Aug. 9-15			(35th) Aug. 30-Sept. 5			(11th) Mar. 15-21		
Total since low.....	10,292,24,960,13,448			26,627,31,102,45,415			29,62,104			3,480,3,609,4,657		

<sup>1</sup> Period ended earlier than Saturday.

<sup>2</sup> Dates between which the approximate low week ends. The specific date will vary from year to year.

<sup>3</sup> Including paratyphoid fever reported separately as follows: Vermont 1; Massachusetts 2 (salmonella infection); Oregon 1.

*Telegraphic morbidity reports from State health officers for the week ended Jan. 17, 1948, and comparison with corresponding week of 1947 and 5-year median—Con.*

Division and State	Whooping cough			Week ended Jan. 17, 1948							
	Week ended—		Median 1943-47	Dysentery			Encephalitis, infectious	Rocky Mt. spotted fever	Tularemia	Typhus fever, endemic	Undulant fever
	Jan. 17, 1948	Jan. 11, 1947		Amebic	Bacillary	Un- specified					
NEW ENGLAND											
Maine.....	13	17	34	—	—	—	—	—	—	—	1
New Hampshire.....	—	—	3	—	—	—	—	—	—	—	2
Vermont.....	51	19	34	—	—	—	1	—	—	—	1
Massachusetts.....	119	196	189	—	3	—	1	—	—	—	6
Rhode Island.....	6	9	18	—	—	—	—	—	—	—	—
Connecticut.....	27	46	83	1	—	—	—	—	—	—	—
MIDDLE ATLANTIC											
New York.....	154	298	298	5	5	—	1	—	—	1	9
New Jersey.....	69	123	123	4	—	—	—	—	—	—	1
Pennsylvania.....	133	221	173	2	—	—	—	—	—	—	2
EAST NORTH CENTRAL											
Ohio.....	107	88	89	1	—	—	—	—	—	—	—
Indiana.....	29	34	23	—	—	—	—	—	—	—	—
Illinois.....	73	104	77	6	3	—	1	—	1	—	13
Michigan <sup>1</sup> .....	134	171	122	2	3	—	—	—	—	—	5
Wisconsin.....	86	149	80	—	—	—	—	—	—	—	1
WEST NORTH CENTRAL											
Minnesota.....	79	6	32	—	—	—	—	—	—	—	5
Iowa.....	16	9	9	1	—	—	—	—	—	—	2
Missouri.....	26	21	13	—	—	—	—	—	3	—	—
North Dakota.....	5	—	4	—	—	—	—	—	—	—	—
South Dakota.....	4	—	2	—	—	—	—	—	—	—	2
Nebraska.....	19	11	3	—	—	—	—	—	—	—	4
Kansas.....	45	8	34	—	—	—	—	—	—	—	—
SOUTH ATLANTIC											
Delaware.....	1	9	3	—	—	—	—	—	—	—	—
Maryland <sup>1</sup> .....	44	80	77	—	—	5	—	—	2	—	—
District of Columbia.....	11	11	9	—	—	—	—	—	—	—	—
Virginia.....	66	31	39	1	—	47	—	—	4	—	1
West Virginia.....	14	36	31	—	—	—	—	—	—	—	—
North Carolina.....	29	67	77	1	—	—	—	1	1	1	—
South Carolina.....	56	66	64	—	—	—	—	—	1	1	3
Georgia.....	26	4	7	1	1	—	—	—	2	3	—
Florida.....	24	32	25	3	—	—	1	—	1	2	—
EAST SOUTH CENTRAL											
Kentucky.....	20	43	38	—	—	1	—	—	1	—	—
Tennessee.....	38	23	23	1	—	2	—	—	2	—	1
Alabama.....	25	28	28	—	—	—	—	—	—	3	2
Mississippi <sup>1</sup> .....	3	—	—	4	—	—	—	—	1	3	—
WEST SOUTH CENTRAL											
Arkansas.....	52	9	9	—	—	1	—	—	—	—	2
Louisiana.....	15	5	2	2	—	—	—	—	2	—	—
Oklahoma.....	38	5	5	—	—	—	—	—	2	—	1
Texas.....	381	240	174	23	301	257	—	—	1	5	10
MOUNTAIN											
Montana.....	10	2	6	—	—	—	—	—	—	—	—
Idaho.....	6	3	2	—	—	—	—	—	—	—	—
Wyoming.....	6	—	5	—	—	—	—	—	—	—	—
Colorado.....	71	9	23	—	—	—	—	—	—	—	3
New Mexico.....	7	—	3	—	—	—	—	—	—	—	1
Arizona.....	29	18	18	—	—	11	—	—	—	—	1
Utah <sup>1</sup> .....	13	12	8	—	—	—	—	—	—	—	3
Nevada.....	—	—	—	—	—	—	—	—	—	—	—
PACIFIC											
Washington.....	43	23	33	—	—	—	—	—	—	—	—
Oregon.....	7	8	10	—	—	—	—	—	—	—	—
California.....	98	57	132	3	4	—	—	—	—	2	9
Total.....	2,328	2,351	2,263	61	320	324	5	1	24	21	91
Same week: 1947.....	2,351	—	—	18	427	124	8	0	43	62	67
Median, 1943-47.....	2,263	—	—	31	405	124	8	0	32	70	69
2 weeks: 1948.....	4,745	—	—	100	741	910	10	2	64	41	161
2 weeks: 1947.....	4,097	—	—	55	749	597	12	1	94	99	153
Median, 1943-47.....	4,097	—	—	54	749	263	14	0	54	142	124

<sup>1</sup> Period ended earlier than Saturday.

<sup>2</sup> 3-year median, 1945-47.

Anthrax: Massachusetts, 1 case; Pennsylvania, 1 case.

Leprosy: California, 2 cases.

Alaska: Scarlet fever 2.

Territory of Hawaii: Influenza 1, measles 1, whooping cough 21.



## WEEKLY REPORTS FROM CITIES \*

City reports for week ended Jan. 10, 1948

This table lists the reports from 60 cities of more than 10,000 population distributed throughout the United States, and represents a cross section of the current urban incidence of the diseases included in the table.

Division, State, and City	Diphtheria cases	Encephalitis, infectious, cases	Influenza		Measles cases	Meningitis, meningococcus, cases	Pneumonia deaths	Pollomyelitis cases	Scarlet fever cases	Smallpox cases	Typhoid and paratyphoid fever cases	Whooping cough cases
			Cases	Deaths								
NEW ENGLAND												
Maine:												
Portland.....	0	0		0		0	2	0	2	0	0	1
New Hampshire:												
Concord.....	0	0		0		0	0	0	0	0	0	
Vermont:												
Barre.....	0	0		0		0	0	0	0	0	0	
Massachusetts:												
Boston.....	0	0		0	188	1	15	0	17	0	0	18
Fall River.....	0	0		0	1	0	5	0	0	0	0	4
Springfield.....	0	0	1	0		0	2	0	1	0	0	6
Worcester.....	0	0		0		0	0	0	10	0	0	
Rhode Island:												
Providence.....	0	0		1		0	2	0	5	0	0	9
Connecticut:												
Bridgeport.....	0	0		0	1	0	0	0	0	0	0	2
Hartford.....	0	0		0		0	3	1	1	0	0	8
New Haven.....	0	0	1	0		1	3	0	0	0	0	6
MIDDLE ATLANTIC												
New York:												
Buffalo.....	1	0		0	1	0	16	0	6	0	0	21
New York.....	10	1	1	5	362	6	92	1	53	0	0	40
Rochester.....	0	0		1		0	2	1	10	0	0	4
Syracuse.....	0	0		0	3	0	2	0	12	0	0	9
New Jersey:												
Camden.....	1	0		0		0	6	0	1	0	0	
Newark.....	0	0	2	0	15	1	10	0	11	0	0	12
Trenton.....	1	0		0	8	0	2	0	0	0	0	2
Pennsylvania:												
Philadelphia.....	1	0	28	0	51	0	39	0	55	0	1	33
Pittsburgh.....	0	0	1	2		2	9	1	1	0	1	6
Reading.....	0	0		0	2	0	1	0	2	0	0	3
EAST NORTH CENTRAL												
Ohio:												
Cincinnati.....	0	0		1	25	1	3	0	17	0	0	2
Columbus.....	5	0		0	70	0	6	0	4	0	0	9
Indiana:												
Fort Wayne.....	1	0		0	2	0	0	0	3	0	0	1
Indianapolis.....	1	0		0	35	0	9	0	6	0	0	4
South Bend.....	0	0		0	4	0	0	0	1	0	0	3
Terre Haute.....	0	0		0	12	1	1	0	2	0	0	
Illinois:												
Chicago.....	0	0		0	375	5	23	1	37	0	0	25
Springfield.....	0	0		0		0	2	0	0	0	0	
Michigan:												
Detroit.....	0	0		1	17	1	9	0	31	0	0	32
Flint.....	0	0		0		0	3	0	6	0	0	
Grand Rapids.....	0	0		0	162	0	0	0	4	0	0	9
Wisconsin:												
Kenosha.....	0	0		0	16	0	0	0	1	0	0	
Milwaukee.....	0	1		0	6	2	5	0	16	0	0	16
Racine.....	0	0		0	22	0	1	0	4	0	0	
Superior.....	0	0		0	1	0	2	0	1	0	0	3
WEST NORTH CENTRAL												
Minnesota:												
Duluth.....	0	0		0	1	0	1	0	8	0	0	21
Minneapolis.....	1	0		0	243	0	4	0	13	0	0	39
St. Paul.....	3	0		0	8	0	3	0	6	0	0	9
Missouri:												
Kansas City.....	1	0	7	0	3	0	11	0	7	0	0	7
St. Joseph.....	0	0		0		0	0	1	1	0	1	1
St. Louis.....	4	1	2	1	5	0	12	0	14	0	2	4

\* In some instances the figures include nonresident cases.

## City reports for week ended Jan. 10, 1948—Continued

Division, State, and City	Diphtheria cases	Encephalitis, infectious, cases	Influenza		Measles cases	Meningitis, meningococcus, cases	Pneumonia deaths	Polio-myelitis cases	Scarlet fever cases	Smallpox cases	Typhoid and paratyphoid fever cases	Whooping cough cases
			Cases	Deaths								
WEST NORTH CENTRAL—continued												
North Dakota:												
Fargo.....	6	0		0	20	0	1	0	0	0	0	
Nebraska:												
Omaha.....	0	0		0		1	3	0	3	0	0	5
Kansas:												
Topeka.....	0	0		0		0	1	0	1	0	0	11
Wichita.....	0	0		0		0	7	0	2	0	0	5
SOUTH ATLANTIC												
Delaware:												
Wilmington.....	0	1		0	9	0	4	0	2	0	0	
Maryland:												
Baltimore.....	1	0		0	1	0	7	0	12	0	0	29
Cumberland.....	9	0		0		0	0	0	0	0	0	
Frederick.....	1	0		0		0	0	0	0	0	0	
District of Columbia:												
Washington.....	0	0		0	44	2	9	0	13	0	0	7
Virginia:												
Lynchburg.....	0	0		0		0	3	0	2	0	0	
Richmond.....	0	0		1		0	6	0	0	0	0	25
Roanoke.....	0	0		0		0	0	0	1	0	0	
West Virginia:												
Charleston.....	0	0		0	1	0	6	1	0	0	0	1
Wheeling.....	0	0		0		0	1	0	1	0	0	
North Carolina:												
Raleigh.....	0	0		0		0	4	0	0	0	0	7
Wilmington.....	1	0		0		0	3	0	0	0	0	
Winston-Salem.....	0	0		0		0	3	0	2	0	0	7
South Carolina:												
Charleston.....	0	0	35	1		0	2	0	2	0	0	4
Georgia:												
Atlanta.....	0	0	31	1		2	6	0	3	0	0	2
Brunswick.....	0	0		0		0	0	0	0	0	0	
Savannah.....	0	0	2	0		0	0	0	2	0	0	3
Florida:												
Tampa.....	3	0		0	12	0	2	0	0	0	0	5
EAST SOUTH CENTRAL												
Tennessee:												
Memphis.....	0	0		1	15	1	12	0	3	0	0	6
Nashville.....	0	0		1		0	5	0	3	0	0	
Alabama:												
Birmingham.....	0	0	3	1		0	4	0	2	0	0	
Mobile.....	1	0	13	2		0	3	0	1	0	0	
WEST SOUTH CENTRAL												
Arkansas:												
Little Rock.....	0	0	5	0	1	0	0	0	0	0	0	
Louisiana:												
New Orleans.....	2	0	1	1		1	7	0	3	0	0	3
Shreveport.....	0	0		0		0	6	0	1	0	0	
Oklahoma:												
Oklahoma City.....	0	0	8	0	1	1	1	0	2	0	0	2
Texas:												
Dallas.....	0	0		0	1	0	4	0	2	0	0	8
Galveston.....	0	0		1		0	1	0	0	0	0	1
Houston.....	1	0		0	19	0	7	0	1	0	0	3
San Antonio.....	0	0		1		0	11	1	0	0	0	
MOUNTAIN												
Montana:												
Billings.....	0	0		0	22	0	0	0	0	0	0	
Great Falls.....	1	0		0		0	2	0	0	0	0	
Helena.....	3	0		0		0	0	0	0	0	0	
Missoula.....	0	0		0		0	1	0	0	0	0	
Idaho:												
Boise.....	0	0		0		0	0	1	0	0	0	1
Colorado:												
Denver.....	2	0	4	0	39	0	1	0	11	0	0	15
Pueblo.....	0	0		0		0	1	0	6	0	0	28
Utah:												
Salt Lake City.....	0	0		0	9	0	2	1	2	0	0	3

## City reports for week ended Jan. 10, 1948—Continued

Division, State, and City	Diphtheria cases	Enecephalitis, infectious, cases	Influenza		Measles cases	Meningitis, meningococcus, cases	Pneumonia deaths	Polio myelitis cases	Scarlet fever cases	Smallpox cases	Typhoid and paratyphoid fever cases	Whooping cough cases
			Cases	Deaths								
PACIFIC												
Washington:												
Seattle.....	2	0	-----	0	4	0	5	2	8	0	0	7
Spokane.....	0	0	-----	0	1	0	1	0	0	0	0	-----
Tacoma.....	0	0	-----	0	59	0	0	0	3	0	0	1
California:												
Los Angeles.....	4	0	668	8	18	2	5	0	17	0	0	22
Sacramento.....	0	0	1	1	1	1	0	0	1	0	0	2
San Francisco.....	0	0	3	0	160	0	12	0	8	0	0	2
Total.....	61	4	817	32	2,076	32	465	12	489	0	5	612
Corresponding week, 1947 <sup>1</sup> .....	90	-----	50	16	687	-----	399	-----	443	0	5	489
Average 1943-47 <sup>1</sup> .....	75	-----	1,428	<sup>2</sup> 51	<sup>3</sup> 1,501	-----	<sup>2</sup> 484	-----	891	0	9	565

<sup>1</sup> Exclusive of Oklahoma City.<sup>2</sup> 3-year average, 1945-47.<sup>3</sup> 5-year median, 1943-47.

Dysentery, amebic.—Cases: Buffalo 1; Chicago 1; St. Louis 1; Atlanta 1; Los Angeles 4.

Dysentery, bacillary.—Cases: Worcester 1; Providence 2; Flint 1; Memphis 1; Los Angeles 3.

Dysentery, unspecified.—Cases: San Antonio 2.

Tularemia.—Cases: Kansas City 1; Nashville 2.

Rates (annual basis) per 100,000 population, by geographic groups, for the 90 cities in the preceding table (latest available estimated population, 33,745,800)

	Diphtheria case rates	Enecephalitis, infectious, case rates	Influenza		Measles case rates	Meningitis, meningococcus, case rates	Pneumonia death rates	Polio myelitis case rates	Scarlet fever case rates	Smallpox case rates	Typhoid and paratyphoid fever case rates	Whooping cough case rates
			Case rates	Death rates								
New England.....	0.0	0.0	5.2	2.6	497	5.2	83.6	2.6	94	0.0	0.0	193
Middle Atlantic.....	6.5	0.5	14.8	3.7	205	4.2	82.8	1.4	70	0.0	0.9	60
East North Central.....	4.7	0.7	0.0	1.4	506	6.8	43.4	0.7	90	0.0	0.0	70
West North Central.....	17.9	2.0	17.9	2.0	557	2.0	85.5	2.0	109	0.0	6.0	219
South Atlantic.....	24.5	1.6	111.1	4.9	110	6.5	91.5	1.6	65	0.0	0.0	147
East South Central.....	5.9	0.0	94.4	29.5	89	5.9	141.6	0.0	53	0.0	0.0	35
West South Central.....	7.6	0.0	35.6	7.6	56	5.1	94.0	2.5	23	0.0	0.0	43
Mountain.....	47.7	0.0	31.8	0.0	556	0.0	55.6	15.9	151	0.0	0.0	373
Pacific.....	9.5	0.0	1,062.8	14.2	384	4.7	36.4	3.2	59	0.0	0.0	54
Total.....	9.5	0.6	126.6	5.0	322	5.0	72.0	1.9	76	0.0	0.8	95

## TERRITORIES AND POSSESSIONS

## Puerto Rico

Notifiable diseases—5 weeks ended January 3, 1948.—During the 5 weeks ended January 3, 1948, cases of notifiable diseases were reported in Puerto Rico as follows:

Disease	Cases	Disease	Cases
Chickenpox.....	18	Syphilis.....	170
Diphtheria.....	163	Tetanus.....	16
Dysentery.....	6	Tetanus, infantile.....	2
Gonorrhea.....	235	Tuberculosis (all forms).....	896
Influenza.....	106	Typhoid fever.....	9
Malaria.....	294	Typhus fever (murine).....	5
Measles.....	416	Whooping cough.....	149
Polio myelitis.....	2		

## FOREIGN REPORTS

### CANADA

*Provinces—Communicable diseases—Week ended December 27, 1947.*—During the week ended December 27, 1947, cases of certain communicable diseases were reported by the Dominion Bureau of Statistics of Canada as follows:

Disease	Prince Edward Island	Nova Scotia	New Brunswick	Quebec	Ontario	Manitoba	Saskatchewan	Alberta	British Columbia	Total
Chickenpox.....		27	1	176	228	43	40	38	24	577
Diphtheria.....		1		19	5	1		6		32
German measles.....				7	8	1	1	6	4	27
Influenza.....		4			4					8
Measles.....		1		528	315	19	5	27	4	899
Meningitis, meningococcus.....					1			1	1	3
Mumps.....		7	1	211	72	10	19	20	8	348
Poliomyelitis.....		2			3					5
Scarlet fever.....		2	4	20	64	2	2	8	2	104
Tuberculosis (all forms).....		5	15	69	16	39	2		22	168
Typhoid and paratyphoid fever.....				4					1	5
Undulant fever.....					1				2	3
Venereal diseases:										
Gonorrhea.....	2	5	11	40	61	15	17	38	31	230
Syphilis.....		10	9	29	25	2	4	3	14	96
Other forms.....									2	2
Whooping cough.....		2		43	34	9	1	19	5	113

### JAMAICA

*Notifiable diseases—5 weeks ended January 3, 1948.*—During the 5 weeks ended January 3, 1948, cases of certain notifiable diseases were reported in Kingston, Jamaica, and in the island outside of Kingston, as follows:

Disease	Kingston	Other localities	Disease	Kingston	Other localities
Cerebrospinal meningitis.....	2	1	Leprosy.....	1	2
Chickenpox.....	6		Tuberculosis.....	52	68
Diphtheria.....	5	3	Typhoid fever.....	9	123
Dysentery.....	4	4	Typhus fever (murine).....	2	
Erysipelas.....	1				

## REPORTS OF CHOLERA, PLAGUE, SMALLPOX, TYPHUS FEVER, AND YELLOW FEVER RECEIVED DURING THE CURRENT WEEK

NOTE.—Except in cases of unusual incidence, only those places are included which had not previously reported any of the above-mentioned diseases, except yellow fever, during recent months. All reports of yellow fever are published currently.

A table showing the accumulated figures for these diseases for the year to date is published in the PUBLIC HEALTH REPORTS for the last Friday in each month.

### Plague

*China—Yunnan Province.*—For the period April to November 1947, inclusive, approximately 770 cases of plague with about 220 deaths had occurred in the western part of Yunnan Province, China.

*Madagascar.*—Plague infection has been reported in Madagascar as follows: December 1–10, 1947, 15 cases, 14 deaths; December 11–20, 1947, 10 cases, 7 deaths.

### Smallpox

*China—Shanghai.*—For the week ended January 3, 1948, 58 cases of smallpox were reported in Shanghai, China.

*India—Calcutta.*—For the week ended January 3, 1948, 329 cases of smallpox were reported in Calcutta, India.

*Tunisia.*—For the month of November 1947, 206 cases of smallpox were reported in all of Tunisia. On December 13, 1947, 15 cases of smallpox were reported in Tunis and suburbs and 34 cases were reported in the interior of Tunisia.

### Yellow Fever

*Belgian Congo—Orientale Province—Bondo.*—On November 20, 1947, 1 fatal case of yellow fever was reported in the region of Bondo, Orientale Province, Belgian Congo, and pathologically confirmed January 7, 1948. The last previously reported case of yellow fever in Belgian Congo was during the year 1944.

## DEATHS DURING WEEK ENDED JAN. 10, 1948

[From the Weekly Mortality Index, issued by the National Office of Vital Statistics]

	Week ended Jan. 10, 1948	Correspond- ing week, 1947
Data for 93 large cities of the United States:		
Total deaths.....	11,313	10,638
Median for 3 prior years.....	10,638	
Total deaths, first 2 weeks of year.....	21,731	20,847
Deaths under 1 year of age.....	822	863
Median for 3 prior years.....	661	
Deaths under 1 year of age, first 2 weeks of year.....	1,547	1,677
Data from industrial insurance companies:		
Policies in force.....	66,844,594	67,231,066
Number of death claims.....	14,153	11,563
Death claims per 1,000 policies in force, annual rate.....	11.1	9.0
Death claims per 1,000 policies, first 2 weeks of year, annual rate.....	8.5	8.4

FEDERAL SECURITY AGENCY  
UNITED STATES PUBLIC HEALTH SERVICE  
THOMAS PARRAN, *Surgeon General*

DIVISION OF PUBLIC HEALTH METHODS

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